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BLISTER CANKER OF APPLE TREES; A PHYSIOLOGICAL AND CHEMICAL STUDY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 246

DEAN H. ROSE

(WITH TEN FIGURES)

Introduction

It is now generally recognized that among the most important problems of plant pathology are those connected with the physiology of diseases whose etiology is already known. It is also recognized that this must be the physiology of the host, of the parasite, and of the two in relation to each other, and, further, that such a comprehensive view of all the factors involved furnishes the only rational approach to an understanding of the principles underlying immunity and disease resistance.

In the present paper are given the results of a physiological study of the destructive disease known as Illinois or blister canker, the etiology of which, including the identity of the causal organism, *Nummularia discreta* (Schw.) Tul., was worked out by HASSELBRING (**22**) in 1902. The work reported here is a continuation of an earlier investigation by the writer (**30**) on the oxidase activity of healthy and diseased bark; in addition there is included an account of the catalase activity and microchemical and macrochemical analyses of both kinds of tissues. Further work is planned on the chemistry of the disease, on the rôle of other enzymes than oxidases, and on the physiology of the fungus itself in pure culture.

The work was done in part at the Missouri State Fruit Experiment Station and in part in the Botany Department of the University of Chicago.

Historical

The problem of oxidation by plant and animal tissues or tissue extracts has been studied by many investigators since the time of the pioneer work by SCHÖNBEIN, the discoverer of ozone. An immense literature has accumulated, for reviews of which the reader is referred to publications by CLARK (14), KASTLE (24), BATTELLI and STERN (5), and ATKINS (3). In this paper only those articles will be cited which bear directly on the problem in hand.

That pathological conditions in plants are often accompanied by increased oxidase activity has been shown repeatedly in recent years. WOODS (35) found greater oxidizing power in the chlorotic portions of tobacco leaves affected with mosaic than in the green portions; this has been confirmed by ALLARD (1) and by FREIBERG (20). SORAUER (31, 32) and DOBY (17), working with leaf-roll of potatoes, found oxidase activity greater in diseased tubers than in healthy ones, although the former makes the point that this greater enzyme activity is to be considered a symptom of the disease rather than the cause. BUNZELL (11), working with the curly-dwarf disease of potatoes, showed by an extensive series of tests that "affected plants have a greater oxidase activity than healthy ones of the same age, both in the juice of their tubers and in the juice of their foliage." Similar results were obtained by BUNZELL (10) in work with curly-top of sugar beets. All 4 of these diseases are of the so-called physiological type, and the question is still unsettled for the last 3 whether the increased oxidase activity is the cause of the disease or merely the result of disturbances due to the real but at present unknown cause.

In the case of diseases whose cause is known the oxidase situation seems to be about the same as for those already mentioned. REED (29) found that the juice of apples affected with bitter rot (*Glomerella cingulata*) has greater oxidase activity than that of sound apples. In his previous work the writer (30) found that diseased apple bark shows greater oxidase activity than healthy bark, and is at the same time less acid. This seems to indicate

that the oxidizing power of a tissue bears some relation to its acidity, a relation which was rendered more probable by the fact that, according to titration and indicator tests, the acidity rises in the Bunzell apparatus during the course of an experiment at the same time that oxidation gradually decreases and finally ceases. The suggestion was made, therefore, that "the gradual slowing down of oxidation in the Bunzell apparatus is brought about in part by the accumulation of oxidation products, probably acetic and oxalic acids in the case of pyrogallol, and not by a using up of the oxidase through chemical combination between oxidase and oxidizable substance." The validity of this theory in the light of later investigation will be discussed in the experimental part of this paper.

Experimental

OXIDASE ACTIVITY

EXTRACTS OF FRESH BARK.—An account will first be given of that part of the work done at the Missouri State Fruit Experiment Station. Extracts of fresh Ben Davis bark were used, prepared as follows: limbs were brought in from the orchard, the bark quickly ground in a meat grinder, and water and toluol added in the proportion of 4.25 cc. of toluol for each 100 cc. of water. The mixture was then allowed to extract at 28–30° C. for 1 hour, with frequent stirring, and filtered through filter paper. The proportions of water and toluol used, assuming that the fresh bark contained 50 per cent water, were such as to make the extracts very nearly equivalent to those prepared for the earlier work (30) with dried bark. All data were corrected to the basis of dry weight determined by weighing and drying samples of the ground bark in triplicate to constant weight in a bath at 95–99° C.

Measurement of the amount of oxidation was made by means of the simplified Bunzell apparatus, using 1 cc. of the extract prepared as just described, and either 4 cc. of a 1 per cent solution of pyrogallol, 0.04 gm. of benzidine, or 2 drops (0.025 gm.) of guaiacol; water was added to make the final volume 6 cc. The various combinations of bark, oxidase reagent, and water were run in duplicate.

After the experiment had been set up in the incubator, 1 hour was allowed for the apparatus and solutions to come to a constant temperature. The manometers were then closed and the solutions mixed. No shaking machine was used, but the apparatus holder was tipped back and forth several times whenever a reading was taken. Allowance for temperature variations was made by running with each experiment a blank containing only water and correcting the others by it.

Table I gives the results of two representative experiments, showing the amount of oxidation of the 3 different reagents by

TABLE I

OXIDATION OF PYROGALLOL, BENZIDINE, AND GUAIACOL BY EXTRACTS OF HEALTHY AND DISEASED BARK; MANOMETER READINGS CORRECTED AGAINST APPARATUS CONTAINING ONLY WATER; TEMPERATURE 28–31° C.

DAY OF TEST	EXTRACT OF FRESH BARK						EXTRACT OF DRIED BARK	
	Pyrogallol		Benzidine		Guaiacol		Pyrogallol	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
	Sample 38*a*	Sample 38*b*	Sample 41*a*	Sample 41*b*	Sample 38*a*	Sample 38*b*		
1.....	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.....	0.71	1.62	0.17	0.82	0.02	0.43	0.26	0.57
3.....	1.38	2.46	0.22	1.21	0.11	1.16	0.53	0.94
4.....	1.70	2.50	0.41	1.76	0.21	1.38	0.77	1.20
5.....	1.83	2.50	0.50	2.07	0.27	1.51	0.86	1.36
6.....	.....	.....	.....	.....	.....	.....	1.07	1.50
7.....	2.12	2.66	0.60	2.31	0.32	1.68	.....	.....
8.....	2.20	2.73	0.74	2.52	0.32	1.91	.....	.....
9.....	2.36	2.86	0.77	2.67	0.35	1.98	.....	.....
10.....	2.44	2.96	0.88	2.93	0.42	2.18	1.51	1.94
Ratio..	1.00 to 1.21		1.00 to 3.32		1.00 to 5.19		1.00 to 1.28	

extracts of both healthy and diseased bark. There are included also data from the earlier paper showing the amount of oxidation of pyrogallol by extract of dried bark. The results indicate that for approximately equal amounts of dry matter the dried bark is considerably less active than the fresh (fig. 1). The decrease is probably due to the drying; this is shown more definitely by data to be presented later. It is to be noted that the oxidase activity of diseased bark is definitely greater than that of healthy bark,

although the ratio between the two is greater where benzidine or guaiacol was used as oxidase reagent than where pyrogallol was used. The writer prefers to follow BUNZELL in using the term oxidase activity or oxidizing power rather than "oxidase." Where the latter term occurs in this paper, it is used only for the sake of brevity, with no intent to imply any fixed notion as to the nature of the agent which brings about the oxidation.

Titration and indicator tests on extracts of fresh bark showed the healthy bark to be more acid than the diseased, exactly as had been shown previously in the work with dried bark. No data

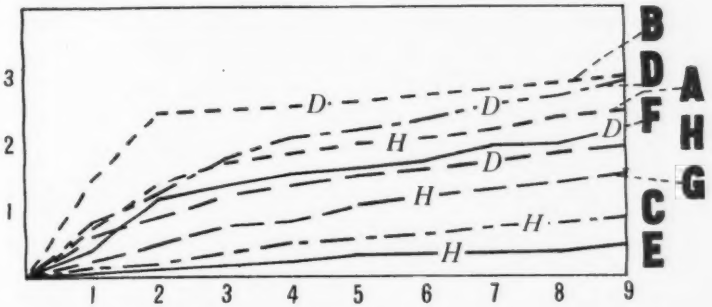

FIG. 1.—Oxidation of pyrogallol, guaiacol, and benzidine by extract of fresh bark, healthy and diseased, and extract of dried bark, healthy and diseased: A, pyrogallol and fresh healthy bark; B, pyrogallol and fresh diseased bark; C, benzidine and fresh healthy bark; D, benzidine and fresh diseased bark; E, guaiacol and fresh healthy bark; F, guaiacol and fresh diseased bark; G, pyrogallol and dried healthy bark; H, pyrogallol and dried diseased bark; *H* = healthy, *D* = diseased.

are given, since the true condition, at least for dried bark, was determined more accurately by means of a potentiometer.

EXTRACTS OF DRIED BARK.—For the work at the University of Chicago bark was used which had been dried at 35–40° C. for 2–3 hours, ground fine enough to go through a 40-mesh sieve, and stored air dry in zinc-capped Mason jars. A few of the experiments were run with oxidases precipitated from an extract of this bark powder, but in most of them the powder itself was used, 0.10 gm. in each apparatus. The reagents tested were pyrogallol and pyrocatechin, 4 cc. of a 1 per cent solution; benzidine 0.05 gm.;

guaiacol 2 drops (0.025 gm.). Tests for any given set of conditions were always run in duplicate, sometimes in triplicate, or even quadruplicate. All experiments were shaken for 3 hours at the rate of 106 complete excursions per minute in a constant temperature chamber provided with a fan driven from the outside, and then allowed to stand for 10–90 hours. Temperature variations were rarely greater than 0.5° during the shaking period, but sometimes amounted to as much as 1.0° afterward, owing to less perfect control when the machinery was not in motion. Corrections for temperature variations were made as before by comparison with a blank containing only water.

Potentiometer measurements were made with a hydrogen electrode like that described by BOVIE (**8**), streaming hydrogen, 3 resistance boxes as described by MICHAELIS (**25**, p. 131), a saturated calomel electrode, a normal element checked against another which had been calibrated by the United States Bureau of Standards, and a Leeds and Northrup dead-beat galvanometer. Hydrogen of high purity from a tank of the compressed gas was run through an electrically heated combustion tube containing platinized asbestos and then through the hydrogen electrode tube. The latter, together with the capillary from the calomel electrode, projected through a rubber stopper into the vessel containing the solution to be tested. Escape of hydrogen was provided for by a third opening in the stopper. An error was undoubtedly introduced here, due to displacement of CO_2 from the solution, in cases where the hydrogen ion concentration was less than 10^{-5} (MICHAELIS, pp. 142–144), but since the only solutions showing this slight degree of acidity were mixtures of bark, water, and pyrogallol for determination of hydrogen ion concentration before any oxidation had taken place, and since all others were found to be more acid, the error is probably negligible. It could have been avoided entirely by using a Hasselbalch shaking electrode had it and the time for using it been available.

Among the first experiments run was one designed to test fully the oxidase activity of healthy and diseased bark when pyrogallol was used as the oxidizing substance. The results, given in table II, are the average of 5 closely agreeing determinations. These results

agree well with those obtained without a shaking machine in showing considerably greater oxidation by diseased than by healthy bark. The ratio between the two, 1.00:2.19, is larger than that found previously (1.00:1.28), the difference probably being due to differences in drying or possibly to the shaking itself.

TABLE II

OXIDATION OF PYROGALLOL BY HEALTHY AND DISEASED APPLE BARK; SAMPLES 3 AND 4; TEMPERATURE 27 C±1.7° C.

TIME OF READING	MANOMETER READINGS, EXPRESSED IN CM. OF MERCURY, CORRECTED AGAINST BLANK CONTAINING ONLY WATER		TIME OF READING	MANOMETER READINGS, EXPRESSED IN CM. OF MERCURY, CORRECTED AGAINST BLANK CONTAINING ONLY WATER	
	Healthy	Diseased		Healthy	Diseased
March 19					
2:45 P.M.	0.0	0.0	March 19	0.48	1.25
3:00	0.0	0.23	4:45 P.M.	0.53	1.33
3:15	0.10	0.48	5:00	0.61	1.45
3:30	0.16	0.65	5:15	0.64	1.57
3:45	0.23	0.80	5:30	0.59	1.49
4:00	0.31	0.92	5:55		
4:15	0.41	1.05	March 20	1.10	2.41
4:30	0.45	1.11	8:30 A.M.		

In table III are summarized the results of an experiment to test the oxidizing power of both diseased and healthy bark on pyrocatechin, guaiacol, and benzidine.

A comparison of the figures in table III with those in tables I and II shows that diseased bark causes greater oxidation of pyrogallol, pyrocatechin, benzidine, and guaiacol than does healthy bark, and that both tissues cause greater oxidation of the first two reagents than of the last two. It is further shown by tables I and III that the amount of oxidation increases slowly for several days; in fact table III shows that it is practically doubled for all the combinations, except those containing pyrocatechin, during the 64-hour period following the 3 hours' shaking. This fact of an increase of oxidation on standing was observed to a greater or less degree with most of the bark material used in this work, and is in direct contradiction to BUNZELL'S explicit and repeated statement that oxidation in his apparatus comes to a definite end after 3 or 4 hours' shaking. The only exceptions the writer has

noted were in those cases where the bark powder showed low oxidase activity to begin with, possibly due to injury of the "oxidase" during drying.

TABLE III

OXIDATION OF PYROCATECHIN, GUAIACOL, AND BENZIDINE BY HEALTHY AND DISEASED BARK; TEMPERATURE 29.4–29.7° C.

TIME OF READING	HEALTHY			DISEASED		
	Benzidine	Guaiacol	Pyrocatechin	Benzidine	Guaiacol	Pyrocatechin
June 8, 1:30 P.M....	0.0	0.0	0.0	0.0	0.0	0.0
4:30 after shaking 3 hours..	0.08	0.33	1.13	0.65	0.75	3.77
June 9, 8:10 A.M...	0.25	0.35	1.45	0.80	1.00	4.35
2:20 P.M...	0.38	0.48	1.65	0.98	1.07	4.55
" 10, 9:15 A.M...	0.40	0.55	1.85	1.27	1.20	4.87
" 11, 8:20 A.M...	0.65	0.65	2.12	1.45	1.47	5.12

That the rate and temperature of drying have an effect on the oxidase activity as well as on the hydrogen ion concentration is clearly shown in table IV.

TABLE IV

EFFECT OF RATE AND TEMPERATURE OF DRYING UPON OXIDASE ACTIVITY AND HYDROGEN ION CONCENTRATION OF HEALTHY AND DISEASED APPLE BARK

SAMPLE	OXIDATION			INITIAL P_H	TEMPERATURE AND DURATION OF DRYING	DEGREE OF BROWNING
	After shaking 3 hours	After standing 10 hours	After standing 15 hours			
4 diseased..	1.49	2.33	2.78	5.61*	40°, 2 hours	Slight
6 " ..	2.25	2.42	.....	5.45	40°, 2	Slight
2 " ..	1.58	1.60	.....	5.16	40°, 4	Much
3 healthy...	0.59	0.80	1.23	5.15	40°, 2	Very little
5 " ...	1.07	1.12	.....	5.04	40°, 2	Slight
5a " ...	0.35	0.35	.....	5.00	50°, 2	Very little
1 " ...	0.62	0.72	.....	4.80	35°, 4	Much

* This figure is the negative logarithmic exponent of 10 where the whole expression $10^{-5.61}$ is a measure of the hydrogen ion concentration in the solution. The larger it is, therefore, the smaller the hydrogen ion concentration it expresses. In this particular case it can be written 2.454×10^{-6} ($6.00 - 5.61 = 0.39$. Antilog $0.39 = 2.454$). In the amplified form this becomes 0.000002454 (normal).

Samples 1, 2, 5, 5a, and 6 were all run in one experiment. Oxidation data for samples 3 and 4 are taken from table II and from another experiment not recorded in this paper. Samples 5 and 5a were parts of the same lot of ground bark but received

different treatments as shown. The results show that oxidase activity is much reduced by drying at 35–40° for 4 hours (sample 1, healthy; sample 2, diseased), or at 50° for 2 hours (sample 5a, healthy).

HYDROGEN ION CONCENTRATION.—Hydrogen ion determinations on mixtures of bark and water and of bark, water, and pyrogallol, used in the same proportions as in the oxidase apparatus, showed that pyrogallol has no effect on the reaction. It was found possible to get constant initial readings on all mixtures containing healthy bark and pyrogallol in 30–45 minutes; the same period sufficed for mixtures containing diseased bark and pyrogallol after they had been shaken in the oxidase apparatus, but not for similar mixtures freshly made up and not shaken. In these cases the potential increased slowly for an hour or two from about $P_H = 5.60$ to $P_H = 5.40$, but never reached the figure given by healthy bark.

CULPEPPER, FOSTER, and CALDWELL (16), working with normal and diseased Red Astrachan apples, state that when titrations were made on fruit pulp suspended in water "the diffusion of acids out of the tissues continues for many hours and at slower rates in diseased than in normal fruits," but in the light of the following results the writer is inclined to think this increase of acidity was due to oxidation going on in the solutions, and not to diffusion of acids out from the tissues.

TABLE V

CORRELATION BETWEEN OXIDASE ACTIVITY AND HYDROGEN ION CONCENTRATION OF MIXTURES CONTAINING PYROGALLOL, WATER, AND EITHER HEALTHY OR DISEASED BARK; TEMPERATURE 29–30.5° C.

STAGE OF EXPERIMENT	HEALTHY		DISEASED	
	Oxidation	P_H	Oxidation	P_H
Before shaking	0.00	5.15	0.00	5.61
After shaking 3 hours	0.82	.....	2.28	.....
After standing 15 hours	1.10	4.82	2.59	4.89
" " 48 "	2.00	.....	4.07	.....
" " 64 "	2.90	4.29	4.96	4.29

INCREASE IN HYDROGEN ION CONCENTRATION DURING OXIDATION.—Experiments designed to test more fully the theory that oxidation causes an increase in acidity are summarized in table V.

It is clear from table V that oxidation in these mixtures is accompanied by a marked increase in hydrogen ion concentration, and the conclusion certainly seems justified that there is a causal relation between the two. It is also seen that when oxidation comes to an end, both mixtures have the same reaction, $P_H = 4.29$, a condition suggesting that at this point the hydrogen ion is the limiting factor.

BUNZELL (12) and REED (28) have studied the effect of hydrogen ion concentration on oxidation, but apparently neither of them has realized that it might increase during the oxidation process (30). They apparently assume that the hydrogen ion concentration established at the beginning of an experiment remains constant until the end, whereas the results given show that in these cases it increased as long as the oxidation continued.

In order to discover, if possible, what relation exists between oxidation and hydrogen ion concentration in the oxidase apparatus, further experiments were tried with mixtures of bark, dry pyrogallol, and, instead of water, 5 cc. of buffer solutions containing various amounts of N/10 sodium acetate and either N/10 or N/100 acetic acid. The initial reactions of these mixtures (before shaking) and of the buffers alone are given in table VI and shown graphically in fig. 2.

TABLE VI

REACTION, P_H, OF BUFFER SOLUTIONS AND MIXTURES OF BUFFER SOLUTIONS, BARK, AND PYROGALLOL

Solution	1	2	3	4	5	6	7	8	9
Buffer alone.....	6.02	5.73	5.41	5.17	4.80	4.53	4.21	3.90	3.61
Buffer and healthy bark and pyrogallol.....	5.59	5.52	5.36	5.15	4.85	4.58	4.24	3.98	3.61
Buffer and diseased bark and pyrogallol.....	5.76	5.70	5.50	5.31	5.00	4.61	4.39	4.08	3.73

Graphs B and C in fig. 2 show that while diseased bark absorbs H^+ ions to about the same extent as the healthy, the latter absorbs more OH^- ions; that is, its titration acidity is greater, which is exactly the condition found by titration with N/20 sodium hydroxide (30). The P_H values at points where B and C cross A

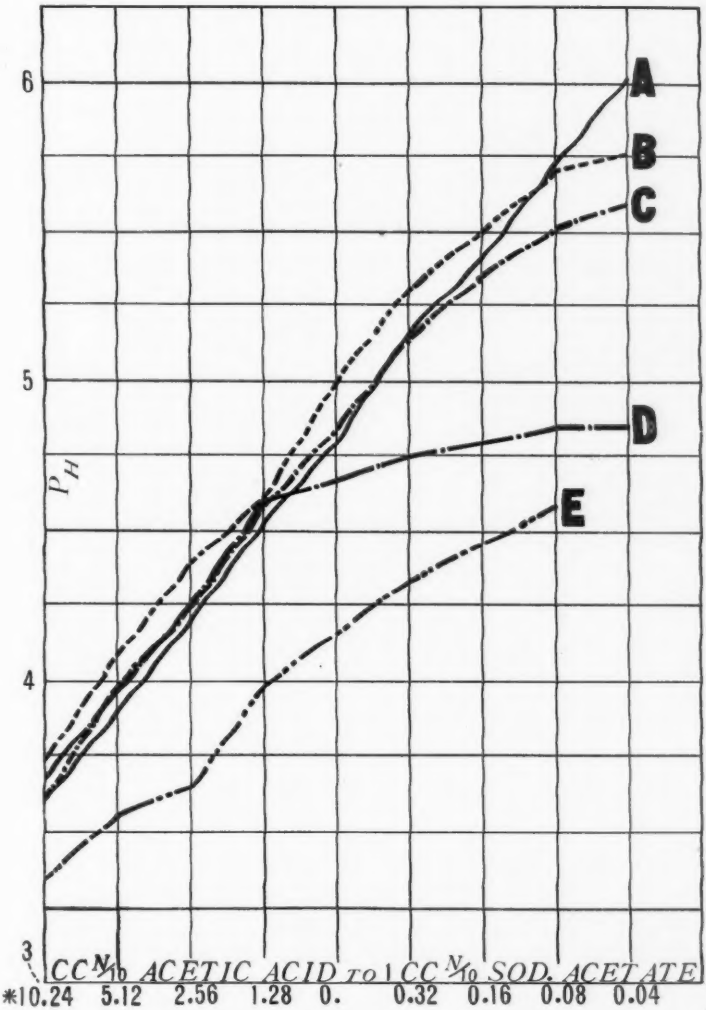

FIG. 2.—P_H of mixtures of bark, pyrogallol, and various buffer solutions before and after oxidation had ceased: A, P_H of buffer solutions; B, P_H of mixtures of buffer solutions, pyrogallol, and diseased bark before oxidation; C, P_H of mixtures of buffer solutions, pyrogallol, and healthy bark before oxidation; D, P_H of mixtures of buffer solutions, pyrogallol, and diseased bark after oxidation; E, P_H of mixtures of buffer solutions, pyrogallol, and healthy bark after oxidation. *Only acetic acid used here.

(healthy bark about 5.10, diseased about 5.65) agree well with those determined without the buffer (P_H healthy = 5.15, diseased = 5.61); the latter are taken, therefore, to represent practically the actual acidity in each case. This is based on the assumption that if the acidity of a buffer solution is the same as that of a mixture of bark, pyrogallol, and water, no change in acidity will take place when the buffer is used instead of water.

EFFECT OF BUFFER SOLUTIONS.—The oxidations brought about by mixtures of bark, pyrogallol, and the various buffer solutions are given in table VII, together with the initial P_H of these mixtures and their P_H after oxidation had practically ceased.

TABLE VII

OXIDATION BY MIXTURES OF BARK AND PYROGALLOL WITH VARIOUS BUFFER SOLUTIONS; TEMPERATURE 29–30° C.

BUFFER SOLUTION	HEALTHY			DISEASED		
	Oxidation	Initial P_H	Final P_H	Oxidation	Initial P_H	Final P_H
1	.....	5.59	.....	4.68	5.76	4.85
2	1.68	5.52	4.58	4.78	5.70	4.85
3	.....	5.36	.....	.....	5.50	.....
4	2.15	5.15	4.34	4.36	5.31	4.75
5	.....	4.85	.....	.....	5.00	.....
6	1.95	4.58	3.98	4.48	4.61	4.60
7	1.80	4.24	3.65	4.12	4.39	4.25
8	1.55	3.98	3.56	.....	4.08	.....
9	0.53	3.61	3.35	1.82	3.73	3.68
Check	2.22	5.15	4.29	4.27	5.61	4.29

The principal fact shown by the results in table VII is that the P_H (4.29) reached by mixtures of pyrogallol, water, and either healthy or diseased bark when oxidation comes to an end is not sufficient to inhibit oxidation when the mixture has that P_H value to begin with; in fact, a greater degree of acidity does not inhibit entirely, since a healthy bark mixture with an initial P_H of 3.61 gave an oxidation (a mercury rise) of 0.53 cm., and a diseased bark mixture with an initial P_H of 3.78 gave an oxidation of 1.82 cm. The check, bark, pyrogallol, and water gave, in the former case, 2.22 cm. mercury rise, and in the latter 4.27 cm.

It might seem from this that the acidity brought about in mixtures of bark, pyrogallol, and water is not the factor which

brings oxidation to an end. It seems more reasonable to suppose, however, that the time factor is of importance here; that is, that an acidity of $P_H = 4.29$ is more effective when reached gradually than when established as a starting point. Looking at the situation from another angle, we may say that inhibition is total if the initial hydrogen ion concentration is high enough, but will be only partial if the concentration is lower; but since partial inhibition means some oxidation, which in itself increases acidity, the process in time necessarily comes to an end. The hydrogen ion concentration at that point will depend on what it was in the beginning, but will never be equal to that which causes total inhibition.

That this theory fits the facts is shown by table VII. Oxidation took place in all the mixtures, the amount depending on the initial hydrogen ion concentration, except where diseased bark was used with buffer no. 4. Acidity increased in all the mixtures but one, diseased bark with buffer no. 6 (see tables VI and VII). The increase in acidity is shown graphically in fig. 2. It is unexpectedly small for diseased bark except where the 3 most alkaline buffers were used, a condition which suggests the need of further experiments.

In figs. 3 and 4 are shown graphically the oxidation data given in table VII, representing the final amounts of oxidation for each set of tests (healthy and diseased bark with the different buffer solutions). In addition there are shown graphs for several earlier stages in each experiment. These graphs show that below 1×10^{-4} ($P_H = 4$) for healthy bark, and 2.5×10^{-5} ($P_H = 4.39$) for diseased bark, oxidation drops rapidly as acidity increases. Above these points the changes are not so marked. The hydrogen ion concentration for total inhibition, estimated by extrapolation to the base line, lies between 3.55 and 3.80×10^{-4} for healthy and between 3.55 and 4.27×10^{-4} for diseased bark. All these figures closely approximate those found by BUNZELL (**12**) for potato oxidase, 2.1–2.8×10^{-4}, and by REED (**28**) for apple oxidase, 5.0–7.0×10^{-4}.

The results given in table VII show that hydrogen ion concentration is not the only factor effective in controlling oxidation in the apparatus, and consequently that the lower hydrogen ion concentration of diseased bark cannot account entirely for its

greater oxidizing power. For example, when both kinds of bark were brought to approximately the same hydrogen ion concentration by buffer no. 6, the final amount of oxidation (mercury rise) for healthy bark was 1.95 and for diseased 4.48, the final P_H 3.98 and 4.60 respectively. The total oxidase activity of the diseased plant is the joint oxidase activity of the host and parasite, while

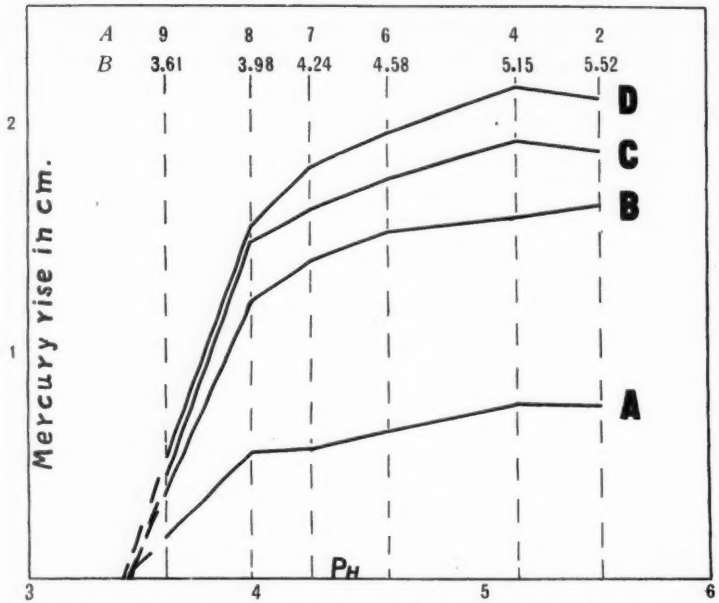

FIG. 3.—Oxidation of mixtures of healthy bark, pyrogallol, and various buffer solutions: A, after 3 hours; B, after 22 hours (19 hours without shaking); C, after 29 hours; D, after 48 hours; *A*, bark, pyrogallol, and buffer solutions as indicated by numbers; *B*, initial P_H; points of plotting marked by vertical broken lines.

the oxidase activity of the healthy plant is that of the host alone. This may account in part for the difference both in rate of activity and in the P_H concentration at the time the action ceases.

NATURE OF EQUILIBRIUM REACHED.—BUNZELL (13), in experiments with potato peel powder, has obtained what he considers evidence that "the activity of the plant powder is not paralyzed by the products formed in the course of the reaction." He found

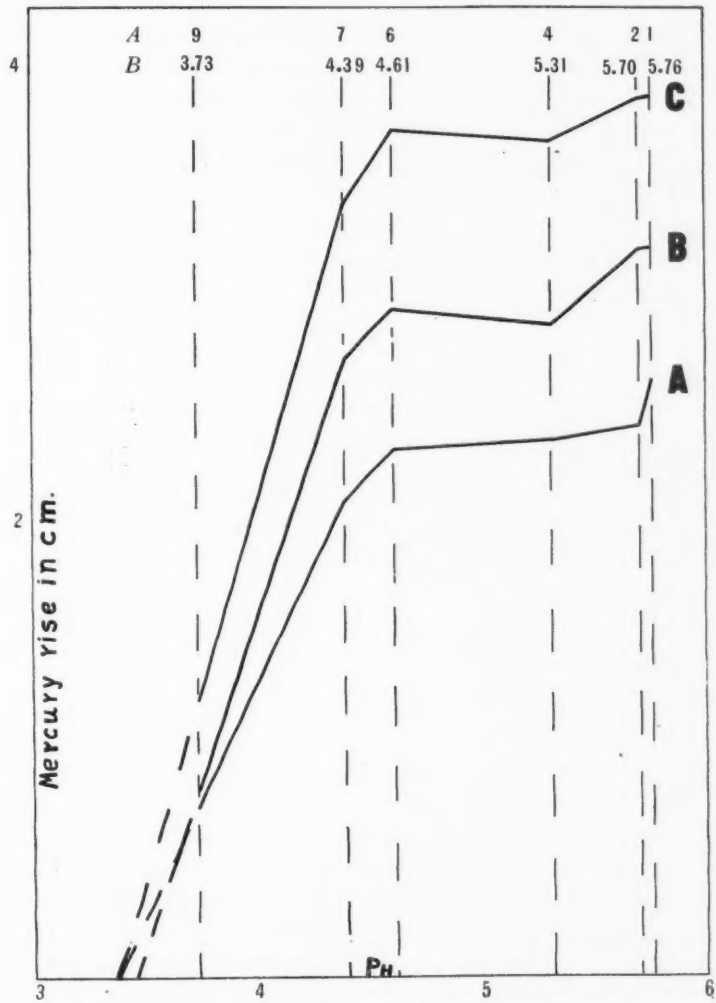

FIG. 4.—Oxidation by mixtures of diseased bark, pyrogallol, and various buffer solutions: A, after 23 hours (shaken 2 hours of this time); B, after 45.5 hours; C, after 69.5 hours; *A* and *B* as in fig. 3.

that by adding a second portion of the powder to the apparatus in which oxidation by the first portion had ceased he could cause a further increase in oxidation, the amount of increase varying with the oxidase reagent used. The writer has found a similar increase in oxidation when more oxidase reagent is added, after oxidation ceases. The results of an experiment of this kind are summarized in table VIII. Results are given beginning with the

TABLE VIII

SUMMARY OF RESULTS FROM AN EXPERIMENT TO TEST EFFECT OF ADDING FRESH SUPPLY OF OXIDASE REAGENT.

Experiment	Stage of experiment	Increase in oxidation (cm. of mercury rise)			
		8	9	10	11
Effect of adding 7 and 4 drops 1 per cent benzidine to apparatus 10 and 11 on ninth day, 8 and 9 as checks	9th to 11th	0.00	0.02	0.49	0.19
Effect of adding 10 drops 1 per cent benzidine to apparatus 10 and 11 on eleventh day, 8 and 9 as checks	11th to 14th	0.17	0.23	1.16	0.71
Total effect of 1 per cent benzidine, 8 as check	9th to 26th	0.47	.....	1.95	1.34
Effect of adding 0.06 gm. of pyrogallol to 8 on twenty-sixth day	26th to 41st	1.53	.....	.....	.....
8 as check	9th to 26th	0.47	.....	.....	.....
Effect of adding 0.06 gm. benzidine to 9 on fourteenth day, 8 as check	14th to 21st	0.19	0.74	.....	.....
Effect of adding 10 drops absolute alcohol to 9 on twenty-first day, 8 as check	21st to 26th	0.11	0.68	.....	.....

ninth day of the experiment. Up to that time oxidation in all 4 of the tubes was practically the same, the average being 3.12 (cm. of mercury rise). Alcohol was used at the beginning of the experiment to discover whether it has an inhibiting effect on oxidation, and later, when solid benzidine was added, to bring the benzidine into solution more rapidly. The results show that, in the small quantities used, the alcohol had no inhibiting effect (table VIII, ninth day, apparatus 10 and 11), and probably did bring the benzidine into solution (twenty-first–forty-first day, apparatus 9).

The most important fact shown by these results is that after oxidation had practically ended, the addition of more oxidase

reagent was followed by a marked increase in oxidation. For example, in table VIII it is seen that from the ninth to the twenty-sixth day oxidation in apparatus 8, containing pyrogallol and bark extract, showed an increase of only 0.47 cm., while tubes 10 and 11, also containing pyrogallol and bark extract to which benzidine solution was added later, showed an increase of 1.95 and 1.34 cm. respectively. Equally marked excess over the check was obtained when solid pyrogallol or solid benzidine was added. One might infer that the oxygen admitted, when the tubes were opened to introduce reagents, increased oxidation, but this effect could hardly account for the difference observed. BUNZELL states that exhaustion of oxygen is not the limiting factor, and experiments by the writer have shown that, when a fresh oxygen supply is allowed to enter the apparatus, the subsequent increase in oxidation is small.

The fact that after oxidation ends it can be started afresh by the addition of fresh plant material or of fresh oxidase reagent suggests that the equilibrium reached is a false one, like the third case described by HÖBER (**23**, p. 671), in which a reaction product of the catalytic reaction brings about equilibrium by an inactivation of the catalyzer. A test for this condition according to HÖBER is that reaction begins again when more catalyzer is added, as in the case of the hydrolysis of amygdalin by emulsin. The similarity between the two reactions, however, does not prove that the oxidation catalyst is an enzyme, for it may be non-enzymic in nature and still be inactivated by the products of the catalytic reaction.

An idea of the nature of the oxidase reaction was obtained by testing some of the data by the formula for unimolecular reaction, $k = \frac{1}{t} \log. \frac{a}{a-x}$. In these calculations the total amount of oxidation (mercury rise) at the end of the shaking period was assumed for the value of a, and the amount of oxidation at the end of each 15-minute interval for the value of x. The figures which should be used, of course, are the total amount of pyrogallol at the beginning of the experiment and the amount oxidized at the end of each 15-minute interval, but such figures would be difficult to obtain. The writer sees no reason why the values used for a and x do not truly represent the course of the reduction.

In most cases the values of k given in these tables are fairly constant and may be considered a strong indication that the oxidase reaction is unimolecular. In table XI, column 3, table XII,

TABLE IX

HEALTHY BARK AND PYROGALLOL

t (min.)	x (mercury rise in cm.)	$a-x$	$k=\frac{1}{t}\log.\frac{a}{a-x}$
15	0.14	0.72	[0.00514
30	0.19	0.67	0.00361
45	0.24	0.62	0.00315
60	0.34	0.52	0.00365
75	0.44	0.42	*0.00415
90	0.49	0.37	0.00407
105	0.54	0.32	0.00409
120	0.63	0.23	0.00477
135	0.68	0.18	0.00503
150	0.74	0.12	0.00570
165	0.79	0.07	0.00660
180	0.86	.....	.....
Mean	.....	.....	0.00433

* Brackets in this and following tables indicate those values of k which were considered in calculating the mean.

TABLE X

DISEASED BARK AND PYROGALLOL

t (min.)	x (mercury rise in cm.)	$a-x$	$k=\frac{1}{t}\log.\frac{a}{a-x}$
15	0.13	1.25	0.00286
30	0.30	1.08	[0.00355
45	0.50	0.88	0.00434
60	0.65	0.73	0.00461
75	0.72	0.66	0.00427
90	0.85	0.53	0.00461
105	0.99	0.39	0.00522
120	1.04	0.34	0.00507
135	1.15	0.23	0.00576
150	1.18	0.20	0.00559
165	1.25	0.13	0.00621
180	1.38	.....	.....
Mean	.....	.....	0.00478

column 1, and table XIV, column 1, the values for k show a gradual increase throughout the experiment, and can scarcely be taken to indicate a unimolecular reaction. Table XII, column 1, however, is checked by tables IX and XII, column 3, the mean value

of k being nearly the same in all 3 cases, although it is doubtful whether a mean for table XII, column 1, is really significant.

TABLE XI

VALUES OF k CALCULATED FROM DATA OBTAINED IN EXPERIMENTS WITH APPLE BARK, K_2CO_3, AND PYROGALLOL

t (min)	k			
	K_2CO_3 and pyrogallol	K_2CO_3 and pyrogallol	Healthy bark, K_2CO_3, and pyrogallol	Diseased bark, KC_2O_3, and pyrogallol
15	0.00747	0.00552	0.00114	0.00525
30	0.00776	0.00803	0.00380	0.00600
45	0.00774	0.00773	0.00431	0.00615
60	0.00785	0.00786	0.00368	0.00604
75	0.00808	0.00819	0.00472	0.00606
90	0.00767	0.00750	0.00492	0.00628
105	0.00765	0.00709	0.00514	0.00642
120	0.00786	0.00890	0.00470	0.00657
135	0.00811	0.00941	0.00633	0.00700
150	0.00768	0.00947	0.00663	0.00678
165	0.00805	0.00936	0.00692	0.00862
180	.....	.....	.....	.....
Mean	0.00781	0.00834	0.00482	0.00635

TABLE XII

VALUES OF k CALCULATED FROM DATA OBTAINED IN EXPERIMENTS WITH APPLE BARK, PYROGALLOL, AND PYROCATECHIN

t (min.)	k			
	Healthy bark and pyrogallol	Diseased bark and pyrogallol	Healthy bark and pyrocatechin	Diseased bark and pyrocatechin
15	.....	0.00458	0.00502	0.00483
30	0.00246	0.00528	0.00429	0.00494
45	0.00277	0.00515	0.00357	0.00510
60	0.00322	0.00515	0.00346	0.00488
75	0.00383	0.00510	0.00416	0.00526
90	0.00493	0.00530	0.00433	0.00536
105	0.00460	0.00507	0.00426	0.00553
120	0.00501	0.00575	0.00434	0.00584
135	0.00584	0.00604	0.00483	0.00613
150	0.00886	0.00744	0.00548	0.00690
165	.....	.....	0.00590	0.00866
180	.....	.....	.....	.....
Mean	0.00430	0.00527	0.00451	0.00521

Confirmation of the results with apple bark is found in table XIII and table XIV, column 2, based on data obtained by

BUNZELL (**9, 13**) with tulip tree leaves and with potatoes, although the mean value of k in all 3 cases is much larger than that found for bark. Attention has already been called to the fact that the data in table XIV, column 1 (also from BUNZELL's work), fail to fit the equation for a unimolecular reaction. The fact of a marked rise

TABLE XIII

VALUES OF k CALCULATED FROM DATA PUBLISHED BY BUNZELL (**9**) FOR POTATO JUICE AND PYROGALLOL

t (min)	k*	t (min)	k†
10	0.0315	10	0.0246
20	0.0266	30	0.0277
30	0.0240	45	0.0199
40	0.0216	60	0.0168
50	0.0244	75	0.0174
60	0.0277	90	0.0233
70	0.0255	105	.....
80	0.0283	Mean	0.0208
90	.....		
Mean	0.0262		

* **23**, p. 29, table VII, columns 1 and 4.
† **23**, p. 26, table II, columns 5 and 7.

TABLE XIV

VALUES OF k CALCULATED FROM DATA PUBLISHED BY BUNZELL (**13**)

t (min.)	k		t (min.)	k	
	Spinach leaves and para-cresol	Tulip tree leaves and phlorhizin		Spinach leaves and para-cresol	Tulip tree leaves and phlorhizin
15	0.00374	0.0124	90	0.01018	.....
30	0.00654	0.0119	105	.....	.....
45	0.00640	0.0133	120	.....	.....
60	0.00940	0.0137	135	.....	.....
75	0.01092	.....	Mean	.....	0.0137

in the value of k toward the end of the experiments with bark may mean that at that point the "oxidase" oxidizes not *constant fractions* but *constant weights* of pyrogallol in a given time (PHILIP **27**, p. 295). The data at hand, however, are insufficient for a verification of this hypothesis.

A unimolecular reaction is one in which the concentration of only one substance is changed. If oxidation of pyrogallol by plant material in the oxidase apparatus be such a reaction, the substance whose concentration is changed is pyrogallol. The "oxidase" then appears as the catalyst, its concentration remaining unchanged during the course of the reaction. Even at that it is not necessarily proved to be an enzyme, since the linear relationship between time and amount of change is also shown in the oxidation of pyrogallol by potassium carbonate.

EFFECT OF ADDING PROTECTIVE COLLOIDS.—BAYLISS (**6**) and PERRIN (**26**) have suggested that the oxidizing enzyme is an active form of the colloidal hydroxide of manganese, iron, or copper, kept in this active state by an emulsion colloid such as gum or albumin, acting as a protective colloid. Tables XV and XVI show the effects of additions of gelatine and gum arabic. Table XV shows that 0.2 per cent gelatine increases considerably the oxidation by healthy bark and only slightly that by diseased bark. Three other experiments with pyrogallol and 2 with pyrocatechin with 0.2 per cent gelatine added showed similar results. The use of 0.8 per cent gelatine with pyrogallol also showed a similar effect. Both 0.2 and 0.8 per cent gum arabic had little or no effect on healthy bark and a slight accelerating effect on diseased bark.

TABLE XV

EFFECT OF 0.2 PER CENT GELATINE ON OXIDATION OF PYROGALLOL BY HEALTHY AND DISEASED BARK; TEMPERATURE 22–24° C.

TIME OF READING	HEALTHY		DISEASED	
	Without gelatine	With gelatine	Without gelatine	With gelatine
May 18, 7:03 P.M.	0.0	0.0	0.0	0.0
10:03 P.M. after shaking 3 hours	0.77	0.81	2.22	2.24
" 19, 8:10 A.M.	0.94	1.32	2.40	2.54
" 20, 9:35 A.M.	1.37	2.26	2.88	3.00
" 21, 8:15 A.M.	1.65	2.74	3.17	3.27

Since gelatine is amphoteric, one might infer that it or its splitting products act as buffers, thus reducing the rate of increase of the hydrogen ion concentration with progress of the oxidation

(fig. 5). Table XVII, however, shows that gelatine has little effect on the hydrogen ion concentration of oxidizing mixtures of either healthy or diseased bark.

PRECIPITATED OXIDASES.—Experiments were run using precipitated "oxidases," prepared as follows: 2 gm. of bark were allowed to extract with 10 cc. of water and 5 drops of toluol for 1 hour; the extract was then squeezed through moist cheesecloth on to coarse filter paper, the beaker washed with five 1 cc. portions of water and the filter paper finally with two more; 50 cc. of 95 per

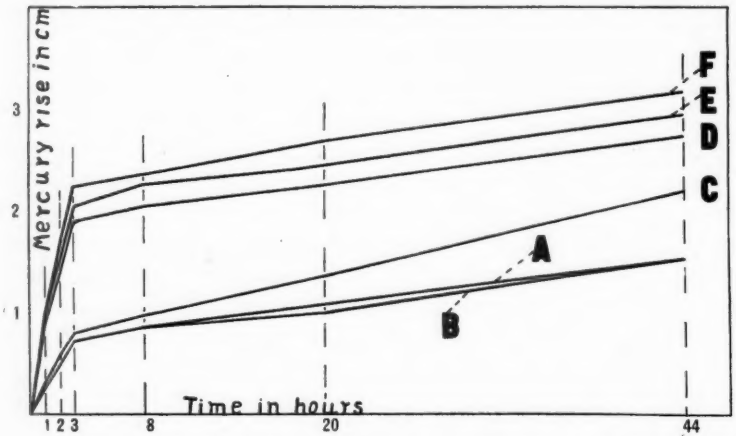

FIG. 5.—Effect of 0.8 per cent gum arabic and 0.8 per cent gelatine on oxidation of pyrogallol by healthy and diseased bark: A, healthy bark; B, healthy bark and gum arabic; C, healthy bark and gelatine; D, diseased bark; E, diseased bark and gum arabic; F, diseased bark and gelatine.

cent alcohol were then added to the filtrate (concentration of alcohol about 70 per cent), the whole allowed to stand for 10 minutes and the flocculent precipitate collected on a hard filter by gentle suction with a filter pump; 150 cc. more alcohol were then added to the filtrate (concentration of alcohol now about 90 per cent) and the whole allowed to stand for 1 hour, since precipitation was slow, before collecting this second fraction on the filter with the first. The precipitate from diseased bark was much browner than that from healthy bark. Whether this bears any relation to its greater oxidase activity is not known.

For tests in the oxidase apparatus the combined precipitates were dissolved in 20 cc. of water, and 2 cc. of this solution containing the precipitate obtained from 0.1 gm. of bark was put in each apparatus together with the usual amounts of pyrogallol and water.

TABLE XVI

EFFECT OF 0.2 PER CENT GUM ARABIC, 0.8 PER CENT GUM ARABIC, AND 0.8 PER CENT GELATINE ON OXIDATION OF PYROGALLOL BY HEALTHY AND DISEASED BARK; TEMPERATURE 21–23° C.

TIME OF READING	HEALTHY				DISEASED			
	No addition	Gelatine 0.8 per cent	Gum arabic		No addition	Gelatine 0.8 per cent	Gum arabic	
			0.2 per cent	0.8 per cent			0.2 per cent	0.8 per cent
At beginning....	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
After shaking 3 hours.....	0.70	0.78	0.65	0.71	1.88	2.23	2.19	2.05
After 18.5 hours	1.03	1.34	1.00	1.01	2.36	2.69	2.71	2.46
After 42 hours..	1.50	2.20	.....	1.54	2.73	3.18	.....	2.95
Average of.....	2	.....	.....	.....	2	.....	.....	.....

In table XVIII are given results showing the oxidizing power of these solutions, with and without gelatine (fig. 6).

The relation observed with bark powder still holds here, that diseased material is more active than healthy. On the other hand, gelatine increases oxidation by the precipitate from extract of

TABLE XVII

REACTION OF MIXTURES OF BARK AND PYROGALLOL WITH GELATINE (0.2 PER CENT) AND WITHOUT AT VARIOUS STAGES OF OXIDATION PROCESS

TIME OF READING		HEALTHY		DISEASED	
		Without gelatine	With gelatine	Without gelatine	With gelatine
P_H	Initial.....	5.15	5.15	5.61	5.60
	After 15 hours.....	4.82	4.84	4.89	4.86
	After 64 hours.....	4.29	4.35	4.29	4.52

diseased bark, but is without marked effect on that from healthy bark, the reverse of the condition found when bark powder was used.

There were indications in the preliminary work that the alcoholic precipitate from bark extract was easily separated into 2

fractions, hence it seemed worth while to collect these separately. This was done for both healthy and diseased tissue and gave

TABLE XVIII

OXIDATION OF PYROGALLOL BY AQUEOUS SOLUTIONS OF PRECIPITATED OXIDASE FROM HEALTHY AND DISEASED BARK, WITH AND WITHOUT GELATINE; TEMPERATURE 29.3–30.3° C.

TIME OF READING	HEALTHY		DISEASED	
	Without gelatine	With gelatine	Without gelatine	With gelatine
June 28, 4:35 P.M.	0.0	0.0	0.0	0.0
" 29, 8:45 A.M.	0.31	0.33	0.68	0.72
" " 11:45 A.M. after shaking 3 hours	0.35	0.46	1.01	1.24
" 30, 8:30 A.M.	0.42	0.46	1.08	1.56

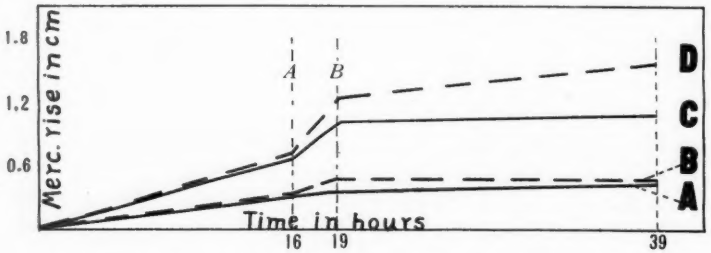

FIG. 6.—Oxidation of pyrogallol by precipitated oxidases from healthy and diseased bark, with and without gelatine, shaken only during period from *A* to *B:* A, precipitate from healthy bark without gelatine; B, precipitate from healthy bark with gelatine; C, precipitate from diseased bark without gelatine; D, precipitate from diseased bark with gelatine.

precipitates whose air dry weights, determined by the use of tared filters, were as follows:

	From extract of healthy bark	From extract of diseased bark
Fraction 1	0.0099 gm.	0.0532 gm.
Fraction 2	0.0080	0.0164
Total	0.0179	0.0696

The greater amount of precipitate from diseased bark may or may not be directly connected with its greater oxidase activity.

Further study is necessary to show the facts. A test of these precipitates with pyrocatechin showed that while the 2 fractions from healthy bark are about equal in oxidizing power the first fraction from diseased bark is 11 times as active as the second (fig. 7).

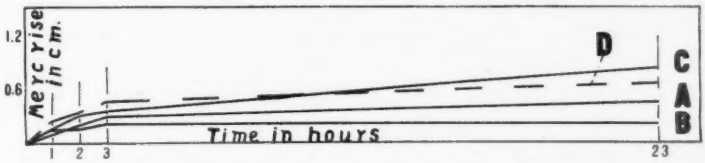

FIG. 7.—Oxidation of pyrocatechin by precipitated oxidases from healthy bark, without gelatine: A, fraction 1; B, fraction 2; C, fractions 1 and 2 tested together; D, sum of fractions 1 and 2 tested separately.

Other precipitates were prepared using 25 cc. of alcohol for the first fraction and 100 cc. more for the second. The oxidase activity of these, tested separately and combined, with and without gelatine, is shown in table XIX.

TABLE XIX

OXIDASE ACTIVITY OF FIRST AND SECOND FRACTIONS FROM BARK EXTRACT TESTED SEPARATELY AND COMBINED; TEMPERATURE 29.5–30.0° C.

BARK EXTRACT	WITHOUT GELATINE		WITH GELATINE	
	Sum of fractions 1 and 2 tested separately	Fractions 1 and 2 combined	Sum of fractions 1 and 2 tested separately	Fractions 1 and 2 combined
Healthy, after 23 hours....	0.65	0.84	0.76	0.84
Diseased, " 38 "	1.82	2.00	2.64	3.06

The mechanism by which gelatine increases the oxidase activity is not clear. It is evidently not through buffer action, as shown by its lack of effect on the hydrogen ion concentration (table XVII, figs. 8, 9, 10). Special tests showed that there was no hydrolysis of the gelatine to amino acids, in either healthy or diseased bark, which would increase its buffer effect. If gelatine is effective through its action as a protective colloid, its effect in this direction must be very complex, as shown by its difference in effect on bark mixtures and precipitated oxidases.

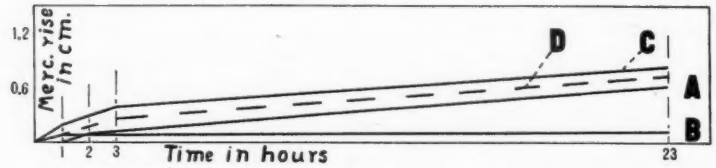

FIG. 8.—Oxidation of pyrocatechin by first and second fractions of healthy bark, with gelatine (for explanation of lettering see legend for fig. 7).

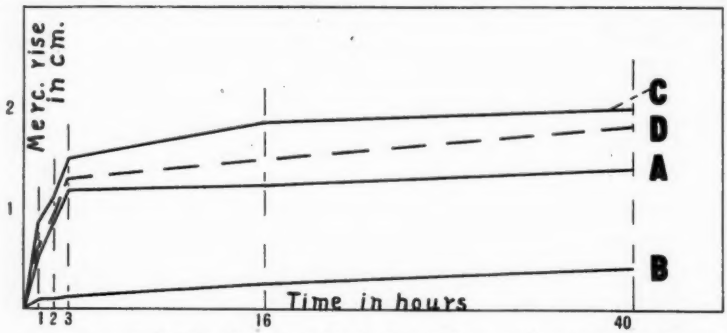

FIG. 9.—Oxidation of pyrocatechin by first and second fractions from diseased bark, without gelatine (for explanation of lettering see legend for fig. 7); points of plotting marked by vertical broken lines.

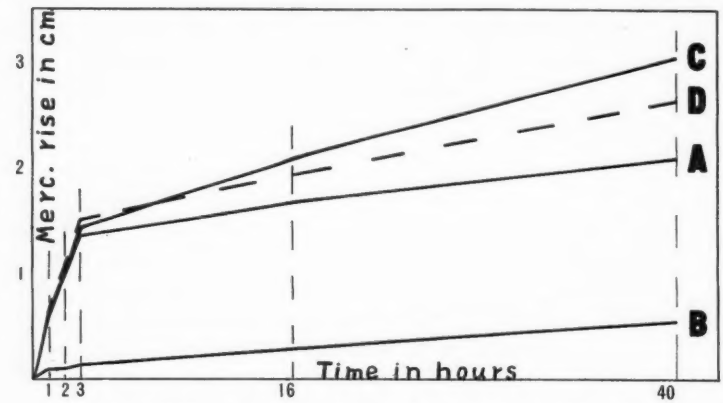

FIG. 10.—Oxidation of pyrocatechin by first and second fractions from diseased bark, with gelatine (for explanation of lettering see legend for fig. 7); points of plotting as in fig. 9.

The difference in the effect of gelatine and of gum arabic on oxidation by healthy bark may depend on differences in the colloidal solutions they form. An artificial oxidase prepared by DONY-HENAULT (**18**) from manganese formate, sodium bicarbonate, and gum arabic could be destroyed by heat; but one prepared by TRILLAT (**33**) from albumin and manganese could not be so destroyed. BAYLISS (**6**, p. 585) thinks the difference here "clearly depends on the nature of the emulsion colloid in association with the metal." On the other hand, what little increase in oxidation gum arabic produces may be due to an oxidase naturally present in it (BOURQUELOT **7**), although an experiment designed to test this question gave negative results. One per cent gum arabic plus 1 per cent pyrogallol, and pyrogallol alone, were placed in separate oxidase tubes and shaken twice during each 24 hours. At the end of 3 days the mercury rise was 0.32 cm. in the first case and 0.20 cm. in the second, a difference almost within the limits of error in reading the manometers.

The data given in table XIX show that when the precipitate is collected in 2 fractions, these fractions have a greater oxidase activity if combined than if used separately. This condition seems to be about the same as that described by BACH and CHODAT (**4**) for *Lactarius vellereus*. They found that by the fractional precipitation of an aqueous solution of the oxidase of this fungus, by alcohol, 2 fractions could be obtained possessing markedly different properties. The first of these was almost insoluble in 40 per cent alcohol and had the properties of a weak oxidase; the second was soluble in 40 per cent alcohol but insoluble in pure alcohol and had no oxidizing powers. This fraction, however, was found to impart greater activity to hydrogen peroxide as an oxidizing agent; it was also found to increase markedly the oxidizing powers of the first fraction. The chief difference between this situation and that found in the work with apple bark is that in the latter case the first fraction has more than a weak oxidase activity, while the second, possibly because of incomplete separation of the fractions, is not entirely without it. No tests have been made of the behavior of the second fraction toward hydrogen peroxide.

OXIDASE ACTIVITY OF THE FUNGUS IN PURE CULTURE.—A fungus powder was prepared according to the method employed by REED (29) from mats of *Nummularia* mycelium grown in the potato extract medium described by DUGGAR (19). A test with 3 Bunzell tubes using 0.1 gm. of fungus powder, 4 cc. of 1 per cent pyrogallol, and 1 cc. of water gave after 4 days an average mercury rise of 2.35 cm. Quantitative tests on the medium in which the fungus had grown showed "oxidase" present there also. From these results it appears probable that the greater oxidase activity of diseased bark is due to a summation of the oxidase activity of normal bark and of the canker fungus itself. This may also account for the difference in behavior of the oxidases of the two.

The general conclusion to be drawn from the preceding data is that diseased bark has greater oxidase activity than healthy bark, probably because of lower acidity and greater degree of dispersion of the oxidizing agent, and because of an actually greater oxidase content. The lower tannin content of diseased bark (see macrochemical work) may also be a contributing factor, since tannins are known to cause inhibition of oxidase action. This factor is probably eliminated when precipitated oxidases are used.

In reference to the Bunzell apparatus it may be said that while it gives valuable comparative measurements of oxidase activity, those using it must realize its limitations. Conditions within it are artificial; with reference to hydrogen ion concentration, and probably other inhibiting factors, they are unstable and continually moving toward an equilibrium which, so far as we know, does not coincide with the equilibrium obtaining in the plant.

Catalase

Determinations of catalase activity (table XX) were made on 12 samples of bark, of which nos. 9 and 10 form a set from one tree and nos. 13 to 20 a set from another tree. Nos. 3 and 4 each came from different trees and are the ones used for most of the oxidase work reported in this paper. They were about 1 year old when tested for catalase. The other samples were freshly prepared for this work in December 1917 and January 1918. The limbs from which they came were carefully cleaned to remove lichens,

Pleurococcus, etc., since microchemical work had shown that such growths have a high catalase activity. The bark was then shaved off, ground in a meat chopper, and allowed to dry on filter paper at room temperature. In the case of samples 9, 10, 14, 16, 18, and 20, calcium carbonate was added during the grinding process at the rate of 0.5 gm. to each 10 gm. of unground bark, to prevent destruction of catalase by the acids of the bark (2) or of the hydrogen peroxide used. The dried bark was finally ground to a powder and only that part used which passed through an 80-mesh sieve.

TABLE XX

CATALASE ACTIVITY OF APPLE BARK

SAMPLE NUMBER	DESCRIPTION OF SAMPLE	TEMPERATURE	POSITIVE PRESSURE IN CM.	
			After 5 min.	After 10 min.
3	Healthy, from sound limb, no carbonate	23.5	0.10	0.10
4	Diseased, no carbonate	.....	0.55	0.95
9	Healthy, from sound limb, plus carbonate	21.0	0.83	1.26
10	Diseased, plus carbonate	.....	5.49	8.59
13	Healthy, from sound limb, no carbonate	21.0	0.52	0.70
14	Healthy, from sound limb, plus carbonate	.....	1.83	3.02
15	Healthy (?) 5 cm. from canker, no carbonate	22.0	0.54	0.65
16	Healthy (?) 5 cm. from canker, plus carbonate	.....	0.73	1.01
17	Diseased, no carbonate	20.5	0.55	0.81
18	Diseased, plus carbonate	.....	1.47	2.74
19	Dead, no carbonate	22.5	5.00	7.37
20	Dead, plus carbonate	.....	7.01	12.17

Tests were made at room temperature by means of the simplified Bunzell apparatus, using 0.03 or 0.10 gm. of bark powder, 1 cc. of water, and 4 cc. of 25 per cent hydrogen peroxide. After the experiment was set up the apparatus was allowed to stand for half an hour, when the manometers were closed and the solutions mixed. The apparatus was shaken for 10 seconds at the end of each minute and readings taken after 5 and 10 minutes. All tests were made in duplicate or quadruplicate, a water blank being included for temperature corrections as in the oxidase work.

A test for catalase was run also on the fungus powder previously mentioned, using 0.03 gm. in each tube and calculating the results to the basis of 0.10 gm. The average mercury rise (positive pressure) produced in 3 tubes was 1.65 cm. in 5 minutes and 2.57 cm. in 10 minutes, or, calculated to the basis of 0.10 gm., 5.49 cm. in 5 minutes, and 8.55 cm. in 10 minutes. It is worthy of note that a powder prepared from *Nummularia* mycelium grown in Raulin's solution, which is acid to litmus, showed no catalase activity. Experiments with different amounts of material showed that the positive pressure varies directly with the amount of material used. It was deemed legitimate, therefore, to calculate all results to the basis of 0.10 gm. of bark powder, and the figures for final tabulation were so calculated.

The results for samples 14, 16, 18, all from the same tree, show that diseased bark (sample 18) had more than twice the catalase activity of seemingly healthy bark 5 cm. away from the canker (sample 16), but only nine-tenths of that of bark from a sound unaffected limb on the same tree (sample 14). Dead cankered bark from this tree (sample 20) had 4 times the catalase activity of healthy bark, 12 times that of seemingly healthy bark next the canker, and nearly 5 times that of diseased bark. In the case of samples 9 (healthy) and 10 (diseased), the results are reversed, since the diseased had a catalase activity nearly 7 times greater than that of the healthy bark. The reason for the discrepancy between these two sets is not clear. The high catalase activity of sample no. 10 can hardly have been due to the presence of lichens, etc., or of an admixture of really dead bark, for precautions were taken when the samples were removed to avoid these sources of error. From the present data the only conclusion that can be drawn is that diseased bark from different trees varies considerably in its catalase activity, and that in general the more completely the bark is destroyed by the fungus the greater is its catalase activity. This condition is probably to be explained by the presence in the diseased bark of considerable amounts of mycelium which, as shown, produces a catalase of its own.

The seemingly healthy bark near the canker when compared with sound and with diseased bark appears to form an exception

in the series. Its catalase activity is less than that of either of the others and seems to be less affected by tissue acids when no carbonate is added. It is possible that near the canker the host's catalase is injured by materials from the fungus, even in advance of actual invasion by the hyphae. The fungus catalase may not appear here at all, but only later in the diseased bark, and in increasing amounts as the amount of mycelium increases.

The oxidase activity of samples 13, 15, 17, 19, together with the catalase activity of samples 14, 16, 18, 20, identical with them except for the addition of carbonate, are given in table XXI.

TABLE XXI

CATALASE ACTIVITY OF APPLE BARK

DESCRIPTION OF SAMPLE	MANOMETER READINGS EXPRESSED IN CM. OF MERCURY USING 0.1 GM. OF BARK POWDER	
	Catalase	Oxidase
Healthy	3.02	1.16
Healthy (?) 5 cm. from canker	1.01	1.47
Diseased	2.50	1.95
Dead	12.17	0.88

It will be seen that there is a gradual increase in oxidase activity from healthy to diseased bark, but a marked decrease in the case of dead bark. The catalase is considerably lower in apparently healthy bark near a canker than in the bark of an unaffected limb, but very much higher in the bark killed by the fungus than in bark from a healthy limb.

Microchemical analysis

Tests for oxidase, peroxidase, and catalase were made on fresh bark, all others on bark preserved in 50 per cent alcohol. The results are given in table XXII.

In making the tests for oxidase (direct action) and peroxidase (indirect action), the brownish purple color due to oxidation of benzidine was found most marked at first, in both healthy and diseased bark, in a zone 2 or 3 cells wide just inside the cork and in the pith rays. Later it came to about the same intensity over

the whole section. Catalase, judging by evolution of gas when H_2O_2 was added, was evenly distributed in all the tissues. Tests with $FeCl_3$ on sections of bark successively farther and farther

TABLE XXII

RESULTS OF MICROCHEMICAL TESTS ON HEALTHY AND DISEASED BARK

SUBSTANCE	REAGENT	REACTION	
		Healthy	Diseased
Cellulose	IKI and 75 per cent H_2SO_4	++	+
Pectin	Ruthenium red	+	+
Lignin	Phloroglucin and HCl	+ in bast	+ in bast
Tannin	Ferric chloride	++	+
Nitrates	Diphenylamine in 75 per cent H_2SO_4		
Fats	Sudan III	+ Especially in parenchyma next to cork	+ Same as for healthy bark
Calcium (crystals)	50 per cent acetic acid	++ Crystals soluble	++ Crystals soluble
	50 per cent H_2SO_4	++ $CaSO_4$ formed	++ $CaSO_4$ formed
Calcium oxalate (crystals)	Oxalic acid	++ Crystals not changed	++ Crystals not changed
Direct reducing sugars	Flückigers reagent	+	++
Starch	IKI	++	+
Cyanogenic glucoside, probably amygdalin	Picric acid and Na_2CO_3	+	
	Berlin blue reaction	+	
Oxidase (direct action)	1 per cent benzidine in 50 per cent alcohol	+	+
Peroxidase (indirect action)	1 per cent benzidine and H_2O_2	+	+
Catalase	H_2O_2	+	+

distant from the badly browned region showed steadily increasing amounts of tannin. Pectin seemed to be present in about equal amounts in both healthy and diseased tissues.

Macrochemical analysis

Six samples were analyzed. The analytical methods used for 4 of them are based on those employed by KOCH for the quantitative study of animal and plant tissues (**21**, pp. 199–207). The difference in material required minor variations from these methods, but it is not thought necessary to describe them here. The other

2 were analyzed according to a method devised by KRAYBILL (unpublished work) in a study of the chemical composition of tomato plants. Material for 4 of the samples, healthy 1 and 2 and diseased 1 and 2, was taken from 8–10 cm. apple limbs cut in January at the Missouri State Fruit Experiment Station, and shipped from there by express. As soon as these samples arrived they were prepared as follows: bark designated as "healthy" was removed from sound limbs with a box scraper and cut into pieces half an inch square; about 150 gm. were then weighed quickly on a torsion balance to hundredths of a gram and put into enough redistilled alcohol (95 per cent) to give an alcohol concentration of approximately 85 per cent. The bottles containing the samples were then set into a steam bath until the alcohol came to a boil, then on top for 1 hour longer, to inactivate the enzymes.

Bark designated as "diseased" was taken from 8–10 cm. limbs showing well developed but not old cankers, usually about 45 cm. long. A strip of moist browned bark 2–3 cm. wide around the outside of the canker was removed with the box scraper, cut up, weighed, and preserved as described. This material usually contained small portions of the seemingly healthy bark outside of the canker, but never any part of the black dead material that often covers the central part of the cankered areas. Healthy samples 3 and 4 were taken from a 7 cm. limb cut in April when the bark peeled easily, to avoid removing small shavings of wood along with the bark, as was inadvertently done in the case of healthy samples 1 and 2 (see discussion of table XXV). Healthy samples 3 and 4 were not extracted with hot alcohol and ether as in the method described by HARVEY (**21**); instead the alcohol for preserving was filtered into a 1000 cc. flask and made up to volume. One-twentieth aliquots were then pipetted off into small beakers, evaporated to a syrup, and used later for dry weight and other estimations. The partly extracted bark was dried as described for the other samples, weighed, ground, allowed to come to air dry condition, and one-twentieth aliquots weighed out as before. This method of handling the material is much shorter than the KOCH method and is very satisfactory if one is not interested in the distribution of substances in the various fractions.

DRY WEIGHT.—One-tenth or one-twentieth aliquots, in tared crucibles or beakers, were brought to constant weight in a vacuum desiccator after intermittent drying for various lengths of time at about 100° C.

NITROGEN.—Estimations were made by the Kjeldahl-Gunning method, modified to include the nitrogen of nitrates. For healthy samples 1 and 2 and diseased samples 1 and 2 estimations were made separately on fractions 2 and 3; no nitrogen was found in fraction 2. Estimations for healthy samples 3 and 4 were made on one-twentieth of the alcohol extract combined with one-twentieth of the partly extracted bark.

CARBOHYDRATES.—Healthy samples 1 and 2, diseased samples 1 and 2: in the case of fraction 2, direct reducing sugars, and reducing sugars after mild hydrolysis, were estimated by the Bertrand volumetric method and calculated as dextrose by use of the MUNSON and WALKER tables (**34**). The more important details of manipulation, including precipitation of non-sugars, are given by CULPEPPER, FOSTER, and CALDWELL (**16**). The polysaccharides in fraction 3 were estimated as dextrose, but after 2.5 instead of 5 hours' hydrolysis (**16**).

Healthy samples 3 and 4: one-twentieth of the air dry, partly extracted bark was further extracted on a filter with about 200 cc. of water at 40° C., the filtrate being collected in a beaker containing one-twentieth of the alcohol extract. Estimation of sugars and polysaccharides in the combined extracts were then made as already described. The results of the analysis are given in tables XXIII and XXIV and summarized in table XXV.

The most important differences shown in the tables, as between healthy samples 1 and 2 and diseased samples 1 and 2, are as follows: diseased tissue contains 3.23 per cent more dry matter than healthy, although here much depends on the manner in which the sample is taken; on the basis of dry weight, fraction 1 is larger in the diseased by 4.56 per cent (nearly doubled), indicating a synthesis of lipoids by the fungus; fraction 3, the alcohol-water-insoluble residue, is larger by 1.83 per cent, while fraction 2, containing the alcohol-water-soluble substances, is smaller by 6.27 per cent. These results are strikingly similar to those found by

CULPEPPER, FOSTER, and CALDWELL (16), working with black rot of apples, caused by *Sphaeropsis malorum*. The increase in total

TABLE XXIII

RESULTS OF ANALYSIS OF HEALTHY BARK

Material	Percentage wet weight	Percentage wet weight	Percentage dry weight	Percentage dry weight
	Sample 1	Sample 2	Sample 1	Sample 2
Total solids	51.55	51.50	.....	.....
" " F_1	2.56	2.59	4.97	5.04
" " F_2	14.84	13.26	28.79	25.85
" " F_3	34.14	35.45	66.21	69.08
Total nitrogen	0.23	0.23	0.45	0.46
Direct reducing sugars	1.58	1.60	3.06	3.13
Reducing sugars after mild hydrolysis	0.55	0.54	1.07	1.05
Reducing sugars after strong hydrolysis F_1 and F_2	1.07	0.22	2.08	1.71
Reducing sugars after strong hydrolysis F_3	7.40	7.56	14.35	14.74
Reducing sugars after strong hydrolysis, total	8.47	7.78	16.43	16.45
	Sample 3	Sample 4	Sample 3	Sample 4
Total solids	46.13	46.24	.....	.....
Total nitrogen	0.217	0.231	0.46	0.50
Direct reducing sugars	0.915	0.949	1.96	2.05
Reducing sugars after mild hydrolysis	0.634	0.662	1.72	1.45
Reducing sugars after strong hydrolysis	7.524	7.189	16.20	16.55

TABLE XXIV

RESULTS OF ANALYSIS OF DISEASED BARK

Material	Sample 1 Percentage wet weight	Sample 2 Percentage wet weight	Sample 1 Percentage dry weight	Sample 2 Percentage dry weight
Total solids	54.29	55.12	.....	.....
" " F_1	4.69	5.77	8.64	10.47
" " F_2	11.52	11.52	21.21	20.89
" " F_3	38.07	37.94	70.16	68.80
Total nitrogen	0.45	0.45	0.83	0.81
Direct reducing sugars	1.42	1.56	2.62	2.83
Reducing sugars after mild hydrolysis	0.66	0.59	1.22	1.07
Reducing sugars after strong hydrolysis F_1 and F_2	0.13	0.38	0.23	0.70
Reducing sugars after strong hydrolysis F_3	8.80	8.84	16.21	16.04
Reducing sugars after strong hydrolysis, total	8.93	9.22	16.44	16.74

nitrogen in diseased bark may be due to fixation by the fungus or to a withdrawal of nitrogen from the surrounding tissue. Further data are necessary before a conclusion can be reached. CULPEPPER, FOSTER, and CALDWELL found protein-nitrogen content of fraction 2 for diseased apples larger than for normal ones, but the total nitrogen for the whole tissue smaller for the former than for the latter.

TABLE XXV

SUMMARY

MATERIAL	AVERAGE PERCENTAGE WET WEIGHT			AVERAGE PERCENTAGE DRY WEIGHT		
	Healthy 1 and 2	Healthy 3 and 4	Diseased 1 and 2	Healthy 1 and 2	Healthy 3 and 4	Diseased 1 and 2
Total solids	51.53	46.19	54.70	.....	.....	.....
" " F_1	2.58	.....	5.23	5.00	.....	9.56
" " F_2	14.05	.....	11.52	27.32	.....	21.05
" " F_3	34.00	.....	37.57	67.65	.....	69.48
Total nitrogen	0.23	0.24	0.45	0.46	0.48	0.82
Direct reducing sugars	1.59	0.93	1.49	3.00	2.00	2.73
Direct reducing sugars after mild hydrolysis	0.55	0.65	0.62	1.06	1.59	1.15
Direct reducing sugars after strong hydrolysis	8.12	7.35	9.08	16.44	16.38	16.59

Results with healthy samples 3 and 4 furnish little of additional interest. They show, however, that as far as total nitrogen and starch are concerned, the small amount of wood in the other 2 healthy samples had no effect on the results. The difference in the case of dry weight and reducing sugars before and after hydrolysis is probably due to the fact that samples 3 and 4 were taken from a limb cut early in the growing season, while samples 1 and 2 were taken from limbs cut in the dead of winter.

Estimation of tannin

The method used was that of LÖWENTHAL, as modified by PROCTOR (**34**, p. 150). Material for analysis was taken from 8–12 cm. Ben Davis limbs cut in November, December, and January. The bark was cut off as already described, ground in a meat grinder, and transferred to a glass moist chamber at once. About 10 gm. were then weighed out and set to boil in 400 cc. of

water as required by the LÖWENTHAL method; at the same time duplicate samples were taken for moisture determination. Whatever may have been the errors introduced by this method, the agreement between duplicates taken for moisture determination was very close in most cases, as is shown in table XXVI.

TABLE XXVI

PERCENTAGE OF DRY MATTER IN DUPLICATE SAMPLES OF VARIOUS LOTS OF BARK ANALYZED

Sample	Duplicate 1	Duplicate 2	Average
Healthy 1	47.89	47.65	47.77
" 2	45.96	45.76	45.86
" 3	46.95	46.86	46.91
Diseased 4	50.44	49.53	49.99
" 5	49.98	49.41	49.69
" 6	49.86	49.73	49.80
Dead 7	56.47	.....	.....
" 8	64.85	64.74	64.79
" 9	76.34	75.83	76.10

The results of the analysis of 9 different samples of bark are shown in table XXVII.

TABLE XXVII

PERCENTAGE OF TANNIN IN HEALTHY AND DISEASED APPLE BARK

Description of sample	Tannin (percentage dry weight)
Healthy	
1	5.16
2. 8–10 cm. from canker	3.64
3. From same tree as 6 and 9	3.38
Average	4.06
Diseased	
4.	2.49
5. From same limb as 2	3.56
6.	2.93
Average	2.99
Dead (from surface of canker)	
7. From same limb as 1	0.25
8.	1.14
9.	1.51
Average	0.97

The LÖWENTHAL method probably determines merely the easily water-soluble tannins, but fails to reach those tied up with

the suberin. If suberin is for any reason more abundant in the diseased bark, an error would thus be introduced which might invalidate any comparisons based on the results obtained. Subject to this possible correction the results shown in table XXVII confirm those obtained in the microchemical analysis; that is, they show a progressive decrease in tannin as the bark is more and more affected by the disease. Healthy bark was found to contain on the average 4.06 per cent of tannin, diseased 2.99, and dead 0.97. If sample 1 healthy, which gave a high figure, and sample 7 dead, which gave a low figure, be eliminated, the averages become healthy 3.51, diseased 2.99, dead 1.33. The figures for samples 3, 6, and 9, all from the same tree, are healthy 3.38, diseased 2.93, dead 1.51. There is undoubtedly a difference between bark from a sound limb and seemingly healthy bark from a limb that is badly cankered. The latter is usually slightly browned throughout when first cut off and rapidly becomes reddish brown on exposure to air. Really healthy bark under such conditions shows only a slight browning.

Whatever the results with apple bark may mean, they are not in agreement with the statement made by KERR (see COOK and WILSON, **15**, p. 26, footnote) that because of the greater stability of tannin and the disappearance of other constituents "all decayed wood and bark give higher tannin contents, no matter what causes the decay." If confirmed by further analyses they would indicate a different relation between host and parasite with reference to tannin in the case of blister canker than obtained in any of the cases studied by KERR. Leaching of tannin may account for the low percentage found in dead apple bark, as suggested by the chemist of the Chestnut Tree Blight Commission (**15**, p. 6) for old cankers of chestnut blight, but can hardly be responsible for the condition found in diseased bark.

Summary

1. Measurements with the simplified Bunzell apparatus show that apple bark attacked by *Nummularia discreta* causes about twice as much oxidation of pyrogallol, pyrocatechin, guaiacol, and benzidine as does healthy bark.

2. The gradual slowing down of oxidation in the Bunzell apparatus is shown to be due, in part at least, to increasing hydrogen ion concentration, brought about by the oxidation process itself. The equilibrium reached in the oxidase apparatus seems to be a false one, which can be disturbed by the addition of either fresh oxidase reagent or plant material. When tested by the formula for a unimolecular reaction, the oxidase reaction gives values for k, which indicate clearly a linear relationship between time and amount of change and suggest that the oxidase is a catalytic agent.

3. The hydrogen ion concentration of diseased bark ($P_H = 5.61$) is definitely less than that of healthy bark ($P_H = 5.15$). Work with buffer solutions shows that this difference is not great enough to account for all of the difference in the oxidase activity of the two kinds of material. When mixtures of the two are brought to the same hydrogen ion concentration by means of buffer solutions, diseased bark still shows greater oxidase activity.

4. The temperature and duration of drying have an effect on the acidity and the oxidase activity of both healthy and diseased bark.

5. Eight-tenths per cent gelatine increases the oxidase activity of both kinds of bark. This may be due to the action of gelatine as a protective colloid which prevents precipitation of the "oxidase." It is not due to buffer action.

6. The concentration of hydrogen ion necessary or complete inhibition of oxidase activity of healthy bark lies between 3.55 and 3.80×10^{-4}; for that of diseased bark between 3.55 and 4.27×10^{-4}.

7. Oxidation in the apparatus comes to an end only after several days instead of after a few hours, as stated by BUNZELL.

8. When the "oxidase" is precipitated in 2 fractions, the first has greater oxidizing power than the second, and the 2 combined have slightly greater oxidizing power than when tested separately.

9. Catalase determinations gave the following results: healthy 3.02 (cm. positive pressure), seemingly healthy 5 cm. from the canker 1.01, diseased 2.74, dead 12.17; results from oxidase determinations for the same stages were 1.16, 1.47, 1.95, 1.85 (cm. negative pressure). These results show some discrepancies, but justify the general statement that the more severely the bark

is attacked by the fungus the greater is its catalase activity, and that catalase activity in part is in indirect ratio to oxidase activity.

10. Microchemical tests indicate, for diseased bark, a partial disintegration of cellulose, a disappearance of cyanogenic glucoside, and a lower content of starch, calcium oxalate, and tannins.

11. Macrochemical analyses show that diseased bark has a higher percentage of dry matter, lipoids, alcohol-water-insoluble residue, and total nitrogen, but a lower percentage of alcohol-water-soluble material than healthy bark. The percentage of carbohydrates in both tissues seems to be about the same. Differences in tannin content are definite but not large. Sound healthy bark contains more than diseased bark and diseased bark more than dead bark from the surface of the canker.

12. The greater oxidase activity of diseased bark is probably due to the combined activity of the oxidases of fungus and host, lower acidity, and possibly to a greater degree of dispersion of the oxidizing agent. The lower tannin content of diseased bark may also be a contributing factor.

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U.S. DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

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MORPHOLOGY OF THE GENUS ACTINOMYCES. II

CHARLES DRECHSLER

(WITH PLATES II–IX)

Taxonomy of species

The obscurity that has surrounded the morphology of *Actinomyces*, besides involving the genus in improbable speculations concerning its phylogenetic relationship to the bacteria, has brought about also a most unfortunate condition in the taxonomy of the many described species. Most of the work on the genus has been done by investigators with bacteriological inclinations, and even where this has not been true, the prevalent view of the nature of these minute plants has lead to the adoption of methods scarcely applicable to mycological research. A discussion of characteristics like the occurrence of endospores, flagella, capsules, sheaths, and involution forms, cannot be regarded as constituting a morphological treatment more satisfactory for species of *Actinomyces* than for species of *Mucor* or *Boletus*.

The dependence of certain biochemical processes, particularly chromogenesis, on definite conditions of nutrition, and the conspicuous differences resulting from comparatively slight changes in the substratum, have long been noted by students of *Actinomyces*, yet descriptions of new species have continued to appear, based largely and often quite exclusively, on these variable activities. Very frequently writers have not compared their organisms with others reported by previous investigators; and in recent years there has been a tendency to disregard altogether the taxonomic contributions of the preceding decades. Moreover, while identities have frequently not been recognized where they existed, in other cases organisms have been supposed to be identical on extremely slight evidence. One of these cases that has led to an unusual measure of confusion is that of GASPERINI'S *Actinomyces chromogenus*. This species was identified by both GASPERINI and ROSSI-DORIA (19) with an organism isolated from the air by the

latter and designated as *Streptothrix nigra*. In culture it was characterized by a dark brown or black discoloration of certain kinds of substrata, a reaction easily obtained on potato agar, for example, and ascribed by LEHMAN and SANO (13) to the production of tyrosinase. Until recently it has been the custom among writers to refer nearly every member of the genus showing a tyrosinase reaction to *Actinomyces chromogenus*, LUTMAN and CUNNINGHAM (14) going so far as to identify this species with the potato scab organism. This practice, which would unite forms so different in appearance and method of development as, for example, *Actinomyces* I and III, regardless of pronounced differences in size and in dextrorse or sinistrorse condition, is not defensible on morphological grounds. KRAINSKY resolved the "chromogenus" complex into 4 species; while WAKSMAN and CURTIS increased the number of derivatives to 8. Of the 17 morphologically distinct saprophytic species figured in this paper, 11 exhibit a tyrosinase reaction; and these represent less than one-fifth of the number of similarly active species which the writer had occasion to examine.

The genus awaits the attention of an investigator in a position to make a comprehensive study involving at least the larger proportion of species existing within wide geographical ranges. The summaries given later, of the more important facts about each species selected for morphological treatment, are not to be regarded as descriptions intended for taxonomic purposes.

ACTINOMYCES I

CULTURAL CHARACTERS.—On glucose agar (0.5 g. peptone, 10.0 g. glucose, 20.0 g. agar, 1000 cc. tap water) nutritive mycelium of individuals smooth, opalescent, more or less confluent; sporulation moderately slow and commencing as a light creamy zone near the periphery; no diffusible stain. On potato agar (decoction of 200 g. peeled potatoes, 2.0 g. glucose, 20.0 g. agar, 1000 cc. water) nutritive mycelium light olivaceous; sporulation moderately abundant, the raised areas where the yellowish gray fructifications are to appear being previously distinguishable by a deep brownish green coloration; guttation never copious, often absent; tyrosinase reaction moderate, but distinct.

MORPHOLOGY.—The development of the erect sporogenous hyphae of this species is strictly successive, and may be followed in the branch *d* in fig. 2, the younger of any 2 hyphae being distinguishable by its attenuated attachment. The partly disrupted chain of spores *d1* here represents the original prolongation of branch *d;* the chain *d2* represents a secondary branch, the spores here being mature but still retaining their spiral disposition without showing indications of disruption; while *d3* represents a tertiary branch, in which septation has not commenced. A similar sequence is illustrated in the succession of derivatives *b1*, *b2*, *b3*, and *b4* from the branch *b*, as well as in the 5 elements *c1–c5* associated with the branch *c*.

A more complex system of fertile hyphae is shown in fig. 1, but the larger fructifications are probably 10 or even 15 times more extensive, and bear many thousands of spores. The species is characterized by close sinistrorse spirals, of 2–6 turns, and 3–4 μ in diameter, which during the later stages of maturation are relaxed, although indications of them may persist in the flexuous or sinuous course of the mature chains of spores. The mature spores are ellipsoidal, 1–2-nucleated, with a distinctly visible wall and a central vacuole of varying size. They measure 1.2–1.4 × 1.4–20 μ, and upon germinating produce 2–4 germ tubes, which early proliferate numerous branches, and show at intervals some dark staining granules.

Isolated 5 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES II

CULTURAL CHARACTERS.—On glucose agar, growth moderate; nutritive mycelium colorless, early covered with a cretaceous or downy aërial mycelium; pigment absent. On potato agar, development of nutritive mycelium moderately rapid; aërial mycelium appearing in scattered areas, first white, later becoming slightly discolored; substratum stained yellow by a soluble pigment; tyrosinase reaction absent.

MORPHOLOGY.—The most conspicuous feature of this species is the extraordinary thickness of the septa (0.3–0.35 μ) associated

with spore production, and their insertion at distensions in the sporogenous hyphae. A comparison of branch *c1* with the younger branch *c2* (fig. 5) corresponding to the conditions shown diagrammatically in figs. 8*b* and 8*c* respectively, shows that the growth in thickness of the hyphae takes place subsequent to the appearance of the septa. After the filament has attained its growth, the septa split along a median plane (figs. 5*a*, 5*c1*, 8*d*), and the 2 halves are drawn apart by the contraction of the delimited protoplasts. Further maturation occurs in the distribution of the deeply staining wall substance, in the strengthening of the peripheral wall, and by an enlargement of the latter, the elimination of the median, hourglass-like constriction of the spore, resulting finally in an approximately cylindrical structure measuring 0.7–0.9×0.8–1.1 μ.

The terminal and the basal spores of each chain retain a somewhat asymmetrical shape, owing to the absence of the massive septum at one of their ends. By an apparently abnormal development, metachromatic granules may be formed in the spores derived from some hyphae, resulting in a condition illustrated in the lowest spore in fig. 8*f*.

The axial filaments are represented by long prostrate hyphae, branching at irregular, long intervals. Septation is confined to the fertile branches. The sterile hyphal portions below the sporogenous terminations taper gradually toward their attenuated attachments. Development and sporogenesis near the axial terminations are successive, and involve the formation of close sinistrorse spirals of 1–5 turns, 3.5–6.0 μ in diameter.

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES III

A. lavendulae Waksman and Curtis

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium slightly yellow on reverse side, central area completely covered with velvety aërial mycelium, first white but gradually assuming a beautiful lavender shade; no soluble stain. On potato agar, growth very profuse; mycelium abundant, changing from white to lavender; guttation moderate to profuse; tyrosinase reaction vigorous.

Identity with *A. lavendulae* established by comparison of cultural and morphological characteristics.

MORPHOLOGY.—The mycelium consists of long prostrate axial filaments, branching rarely except at the end. Sporulation is usually initiated at the tip of the filament, and proceeds basipetally by the insertion and transformation of almost invisible septa, to the point of attachment of the first sporogenous branch (figs. 10, 11). The sporogenous branches are rarely crowded, although at the base of the sporogenous axial termination an opposite arrangement is not uncommon. Secondary branching occurs frequently; septa are entirely absent, except when associated with the progressive basipetal delimitation of spores.

The sporogenous hyphae terminate in dextrorse, moderately compact spirals of 4–12 turns, 2.0–3.8 μ in diameter. The spores are ellipsoidal, with nuclei not readily demonstrable. Metachromatic material occurs abundantly in many old hyphae (fig. 15).

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES IV

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium colorless; aërial mycelium moderately profuse, velvety in appearance, changing from white to smoky blue; no guttation. On potato agar, tyrosinase reaction vigorous; aërial mycelium first produced white, not subsequently much discolored, becoming matted to the substratum as a result of excessive guttation, and later completely overgrown by a loose growth of smoky blue secondary mycelium.

MORPHOLOGY.—The sporogenous branches with dextrorse spirals of 2–12 open turns, 1.5–2.5 μ in diameter, are attached to the long axial filaments usually at wide intervals, in a loose racemose arrangement. Secondary branching, although rare, occurs occasionally, and is then associated with simultaneous sporulation (fig. 18). Development of the 1–2-nucleated spores, 0.7–0.8×0.9–1.1 μ, proceeds by the insertion of conspicuous septa, followed by their constriction and subsequent conversion to hyaline isthmuses (figs. 70*a–e*). Two germ tubes are usually produced, of a more or

less uniform diameter, and proliferating branches at relatively wide intervals.

Isolated twice from soil collected in Cambridge, Massachusetts.

ACTINOMYCES V

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium on reverse side slightly yellowish; the surface completely covered with a luxuriant velvety or cottony weft of pinkish-yellow aërial mycelium; guttation slight. On potato agar, nutritive mycelium chocolate-colored, firm lichenoid, crimped around margin; aërial mycelium, as on glucose agar, but less profuse; tyrosinase reaction vigorous.

MORPHOLOGY.—The fertile hyphae, which are attached to prostrate axial filaments at long intervals, are terminated by relatively close sinistrorse spirals of 4–12 turns 2.0–4.0 μ in diameter, developing spores (0.6–0.8×0.9–1.1 μ), like *Actinomyces* IV, by the insertion of conspicuous septa, followed by their constriction and conversion. A peculiar characteristic is found in the sterilization of the basal portion of the fertile hyphae, by an apparent abortion of its lower potential spores.

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES VI

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium colorless, completely covered with a felty aërial mycelium, first white, later assuming a deep smoky tinge. On potato agar, nutritive mycelium excessively wrinkled, partially covered with a slightly discolored aërial mycelium; tyrosinase reaction vigorous.

MORPHOLOGY.—The species appears closely allied to *Actinomyces* V, differing from the latter chiefly in the absence of any evidence of sterilization, and in the shorter length of its sinistrorse sporogenous spirals, which consist of only 2–6 turns, 2.0–4.0 μ in diameter. The spores are uninucleated, measure 0.7–0.8×0.9–1.1 μ, and are developed by the insertion and transformation of conspicuous septa. Fertile hyphae are attached to the axial fila-

ments with considerably greater frequency, and secondary branching, characterized by the successive type of development, is common.

Isolated once from soil collected in Cambridge, Massachusetts.

ACTINOMYCES VII

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium colorless, early developing an aërial mycelium from the center outward, the latter changing from white to light gray with increasing age. On potato agar, nutritive mycelium luxuriant, developing rapidly; aërial mycelium represented by a slight cretaceous development toward the top of the slant; tyrosinase reaction vigorous.

MORPHOLOGY.—This species departs from the main trend of the 3 preceding forms in the relatively close arrangement of its branches on the axial filament, and in the elaboration of these branches by further ramifications in a typically successive order. Nearly spherical to ellipsoidal spores, 0.6–0.8×0.7–1.0 μ, are produced from moderately close sinistrorse spirals of 3–8 turns 2.0–3.0 μ in diameter, by the development represented in fig. 70*a–e*, but the septa are relatively thin, and occasionally fall below the limit of clear visibility.

Isolated twice from soil collected in Cambridge, Massachusetts.

ACTINOMYCES VIII

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium nearly colorless, secreting a diffusible yellow pigment; aërial mycelium moderately profuse, velvety, first white, later changing to a light bluish color. On potato agar, growth similar, but soluble pigment absent; no tyrosinase reaction.

MORPHOLOGY.—The fertile hyphae of this species may be attached to the axial filaments in a diffuse racemose arrangement (fig. 46), or crowded in a compact capitate system. The swellings in the axial filaments in figs. 43, 44, 45, and 46 at the bases of sporogenous branches indicate the mode of origin of the *Leptomitus*-like distensions shown in figs. 47 and 48.

The small, ellipsoidal, uninucleated spores, 0.5–0.6×0.6–0.8 μ, are formed from close, sinistrorse spirals of 2–10 turns 1.2–2.5 μ in diameter. Indications of septa can be seen only rarely. The mature spore chains upon collapsing cohere in irregular zoogloœa-like masses, a peculiarity of behavior dependent probably on a gelatinization of wall material. Upon germination, 1 or 2 tubes are produced, relatively thick and abundantly branching.

Isolated 6 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES IX

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium colorless, forming no soluble pigment; aërial mycelium at first white, becoming light smoky blue in the course of a few days. On potato agar, cultural characters similar, but growth more profuse, guttation moderate, discoloration of aërial mycelium more rapid; tyrosinase reaction absent.

MORPHOLOGY.—The most characteristic feature of this species is the greater thickness of the fertile hyphae below the second turn of the spiral. The latter are sinistrorse, usually with very close turns, varying in number from 1 to 16, and measuring 1.5–2.0 μ in diameter. They give rise to ellipsoidal, uninucleated spores, 0.5–0.7×0.6–1.0 μ, without the appearance of clearly visible septa. It seems highly probable that cross-walls nevertheless occur, since occasionally a median partition may be differentiated in the hyaline attentuated connections between two spores (fig. 51), suggesting a development similar to that indicated in fig. 70*a–e*.

Isolated once from soil collected in Cambridge, Massachusetts.

ACTINOMYCES X

Streptothrix alba Rossi-Doria; *Actinomyces griseus* Krainsky (?)

CULTURAL CHARACTERS.—On glucose agar, growth poor and not characteristic. On potato agar, growth excessively rapid, nutritive mycelium colorless; aërial mycelium firm, white, changing rapidly to a yellowish gray; secondary growth occurring in the formation of numerous successive rings of sporodochia, or in the development of cottony white masses of mycelium from below the thick

crust of old mature spores; tyrosinase reaction absent; substratum stained a faint greenish yellow in old cultures.

MORPHOLOGY.—According to WAKSMAN and CURTIS, the aërial filaments of this species possess only a slight tendency to branch. The writer was led to a somewhat different conclusion, as the axial hyphae are usually found to proliferate fertile branches at moderately close intervals. Occasionally, as in fig. 58, indications of a successive sequence may be observed, but more frequently the development of the different elements of a ramifying system occurs without any recognizable interrelation. The short, cylindrical spores, 0.7×0.7–$1.0\ \mu$, are formed, as in *Actinomyces* XVI, by a septation of the fertile hyphae, followed by splitting of the partitions along a median plane, but the septa are usually less conspicuous, and often not clearly visible, and the fertile hyphae show no indication of a spiral condition.

A striking dimorphism characterizes the mycelium of this species, as well as that of a number of other forms observed by the writer. The deeper sterile aërial hyphae below the sporogenous layer typically are extremely minute, with a diameter frequently not exceeding $0.3\ \mu$; their protoplasmic contents show little affinity for stains; and the contours of their walls are uniformly smooth. The more superficial hyphae, which usually attain a thickness of $1.0\ \mu$, and are distinguishable by markedly irregular contours, contain dense deep staining protoplasm; and when septa are present, they are sometimes associated, as in *Actinomyces* XVII and XVIII, with spherical structures. The thicker filaments bear the sporogenous branches, and, in general, appear to constitute the expanded prolongations of the minute hyphae (fig. 59).

Isolated twice from soil collected in Cambridge, Massachusetts; once from tap water; very frequently from outdoor air; several times from gross cultures of dead leaves; 4 times from horse dung undergoing fermentation at 50–60° C.

SYNONOMY.—In his description of *Streptothrix alba*, ROSSI-DORIA records two characteristics that establish its identity beyond much danger of confusion: a conspicuous preponderance in number over any of its congeners on plates exposed to the air, and a tendency toward the formation of concentric rings more pronounced than that of any other species. ROSSI-DORIA

attributed this preponderance in the air to its omnivorous character, enabling it to develop on a large variety of substrata, "Questa *Streptothrix* cresce, si può dire, dappertutto, tanto su terreni di natura vegetale quanto su terreni di natura animale. E per ciò nonchè la grande sua produzione di spore, che essa si trova cosi diffusa nell'aria ed altrove. Pare che essa possa svilupparsi anche nel terreno." In spite of this fortunate and quite distinctive characterization, the specific term "albus" subsequently came to be used in a manner as miscellaneous as "chromogenus," being applied generally to any type with a light mycelium showing no tyrosinase reaction.

The same species was treated in the publication of WAKSMAN and CURTIS as *Actinomyces griseus* Krainsky. I have not been able to satisfy myself fully about the identity of KRAINSKY'S organism; nor would it seem possible to reach any definite conclusion without an examination of authentic material.

ACTINOMYCES XI

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium first colorless, becoming slightly reddened with increasing age; aërial mycelium first white, rapidly changing to a bluish violet. On potato agar, nutritive mycelium gradually becomes deep red by the slow accumulation of a slightly diffusible pigment; tyrosinase reaction absent.

MORPHOLOGY.—More or less erect fructifications are developed along the distal portions of long prostrate filaments. Branching is abundant and only occasionally shows indications of a successive sequence. The aërial hyphae in the dendroidic structures (figs. 64, 66) are often conspicuously vacuolate, especially in the inflated distensions from which a number of fertile branches arise. The latter terminate in sinistrorse spirals of 4–6 turns, 2.0–3.0 μ in diameter, from which, by the insertion of conspicuous septa and their subsequent transformation to hyaline isthmuses, spores 0.5–0.7 × 1.0–1.2 μ are produced.

Isolated once from soil collected in Cambridge, Massachusetts; identical with an organism isolated by Mr. H. J. CONN from soil collected near Geneva, New York.

ACTINOMYCES XII

A. aureus Waksman and Curtis

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium yellowish on reverse side; aërial mycelium changing from white to pale yellowish gray; soluble stain absent. On potato agar, nutritive mycelium darker on reverse side; aërial mycelium more profuse, forming a somewhat more deeply colored felty layer; tyrosinase reaction moderate. Identity with *Actinomyces aureus* established by comparison with authentic material of the latter.

MORPHOLOGY.—In this species long prostrate filaments terminate in more or less erect fructifications. Secondary branches are proliferated from the lateral elements, generally in successive sequence. A more or less pronounced cuneate thickening of the hyphae below the insertion of a branch is characteristic of the species. The ellipsoidal, uninucleated spores, 0.5–0.7×0.8–1.2 μ, are formed by the insertion of conspicuous septa in open, sinistrorse spirals of 2–7 turns, 3.0–4.0 μ in diameter.

Isolated twice from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XIII

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium light orange-brown, the separate individuals fused into a massive pellicle with a depressed, crimped margin. On potato agar, nutritive mycelium dark chocolate-brown, wrinkled, lichenoid, secreting a diffusible red pigment; tyrosinase reaction absent. Aërial mycelium on both substrata loose, cottony; developing slowly, first white, later changing to a dull bluish tint.

MORPHOLOGY.—The aërial mycelium consists of extremely long filaments, which rarely show any evidence of branching (figs. 74 75), and toward their terminations follow an undulating or slightly spiral course. Sporulation occurs as the result of protoplasmic contractions without the appearance of visible septa, the chains of cylindrical spores, 0.4×1.2–1.6 μ, being held together for some time by the evacuated portions of hyphal wall, that seem to undergo no apparent constriction.

Isolated 3 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XIV

CULTURAL CHARACTERS.—On glucose agar, nutritive mycelium usually colorless, but frequently becoming deep brown or black; aërial mycelium consisting of a dense velvety weft, first white, later changing to a creamy yellow. On potato agar, growth similar; tyrosinase reaction absent.

MORPHOLOGY.—This species is characterized by the production of extensive prostrate fructifications through the proliferation of numerous lateral branching processes from long axial filaments (figs. 76, 79, 81). A septum is occasionally present immediately above the attachment of a branch, but more frequently is absent. Secondary ramifications, resulting in more or less complex elements, take place without reference to the stage of sporogenesis in the proliferating branch. The ellipsoidal uninucleated spores, 0.5–0.7×0.8–1.2 μ, are derived from sinistrorse spiral hyphae of 1–8 turns, 2.0–4.0 μ in diameter, by the insertion and transformation of relatively thin septa, or without the appearance of demonstrable septa.

Isolated 4 times from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XV

CULTURAL CHARACTERS.—On glucose or potato agar, nutritive mycelium opalescent; aërial mycelium first white, becoming only slightly discolored with age; tyrosinase reaction moderate.

MORPHOLOGY.—Microscopically this species closely resembles *Actinomyces* IV, differing from the latter chiefly in the abundant proliferation of branches of the second or of a higher order. The lateral elements thus formed follow the successive type of development (figs. 82, 83). The uninucleated spores, 0.7×0.9–1.0 μ, are formed from dextrorse spiral hyphae of 3–12 turns, 1.8–2.5 μ in diameter, by the constriction of conspicuous septa, and their transformation into hyaline isthmuses.

Isolated twice from soil collected in Manhattan, Kansas.

ACTINOMYCES XVI

CULTURAL CHARACTERS.—On glucose agar, growth very meager; never producing an aërial mycelium. On potato agar, develop-

ment rapid; nutritive mycelium dark brown or greenish brown; aërial mycelium profuse, changing from white to violet or pinkish gray; guttation profuse; tyrosinase reaction moderate.

MORPHOLOGY.—In this species the characteristic development consists in the proliferation of a number of long branches in an irregular whorl from a long and somewhat thickened axial filament. Secondary branching is common, but usually more or less remote. Vacuoles associated with hyphal distensions are found in the axial filaments and in the main branches, and metachromatic granules occur abundantly in many of the older sterile hyphae (fig. 91). The long cylindrical spores, 0.6–0.7×1.0–2.0 μ, are formed by the septation of sporogenous hyphae that terminate in open, sinistrorse spirals of 2–3 turns, 4.0–5.5 μ in diameter, followed by the splitting of the septa along a median plane, and the separation of the two halves by a contraction of the delimited protoplasts. The progress of sporogenesis is usually basipetal, but not infrequently the first divisions may result in a number of segments of varying lengths, which by subsequent divisions are reduced to the magnitude of the ultimate spores.

Isolated once from soil collected in Cambridge, Massachusetts.

ACTINOMYCES XVII

A. scabies (Thaxter) Güssow (6)

MORPHOLOGY.—The aërial mycelium of this species, which is one of the largest of dextrorse forms, consists of long prostrate filaments on which lateral branches are inserted at short intervals. Secondary branching is abundant and usually associated with a successive order of development (figs. 93*a1–a3*). The more or less cylindrical spores, 0.8–0.9×1.3–1.5 μ, are developed from dextrorse spiral hyphae of 3–14 turns, 2.0–3.5 μ in diameter, by the insertion of conspicuous septa and their subsequent splitting along a median plane. In many hyphae the septa before their division can be seen to occupy a transverse equatorial position in the peculiar spherical structures to which reference has been made elsewhere, and which here occupy slight but perceptible hyphal distensions (figs. 92, 93*h1*, 93*h2*, 101*cy*). Whenever the spherical structures

are absent, the fertile hyphae are uniformly isodiametric. It is not certain whether these structures appear in all sporogenous branches at some time preceding the contraction of the delimited protoplasts, or are more or less accidental in their occurrence. They also are found associated with septa in the sterile axial filaments, and here similarly occupy local hyphal distensions. After the individual spores have become separated, the connecting segments of evacuated hyphal wall contract slightly to form somewhat narrowed isthmuses, which persist until the mature spore chains are disrupted. In germinating, the spore usually produces 1 or 2 germ tubes.

The preparation from which figs. 92–101 were drawn was derived from one of 5 organisms communicated by Mr. M. SHAPAVALOV, who writes that "all were tested in inoculation experiments in 1912–1913, and proved to be pathogenic." Three of the other organisms were found to be identical morphologically with the one figured in plate VIII, while the fifth did not produce an aërial mycelium sufficiently profuse to permit of a satisfactory microscopic examination, although the general appearance of the culture indicated that it also is identical with *Actinomyces* XVI.

ACTINOMYCES XVIII

CULTURAL CHARACTERS.—On glucose agar, growth meager; nutritive mycelium colorless; aërial mycelium slow to develop, first white, later showing slight discoloration; diffusible pigment absent. On potato agar, development very rapid; nutritive mycelium dark; aërial mycelium profuse, felty, bluish gray; guttation moderate; tyrosinase reaction vigorous.

MORPHOLOGY.—This species is characterized by an unusual degree of variability in its fructifications. In figs. 102 and 107 is represented a relationship between axial filament and sporogenous branches common to many members of the genus. Fig. 108 shows a slight departure from this type in the thickening of the subterminal portion of the axial filament bearing the spiral branches. Further departures are expressed in the tufted grouping of the spiral hyphae in fig. 104, and in the distended and extremely vacuolated condition of the axial filament in fig. 106. A strikingly

aberrant type is seen in fig. 103, the fertile branches being short, inserted at close, irregular intervals, and showing no spiral tendency; while the axial filament is thick and abounding in spherical structures containing either deposits of metachromatic material or a partial equatorial septum.

In the dextrorse spiral hyphae of 1–8 open turns, 2.0–3.0 μ in diameter, the ellipsoidal spores, 0.8–0.9×1.0–1.6 μ, are produced by the insertion of conspicuous septa, sometimes in association with spherical structures. The presence of the latter (fig. 106), however, is not here indicated by local distensions. Subsequently the cross-walls undergo constriction and conversion to narrow connecting isthmuses. In the aberrant fertile hyphae (those without any spiral tendency), sporogenesis appears to take place in a more miscellaneous manner. Definite septa can rarely be distinguished, the spores seeming to result from protoplasmic contractions.

Isolated once from soil collected in Cambridge, Massachusetts.

Summary

1. The vegetative thallus of *Actinomyces* consists of a mycelium composed of profusely branching hyphae, the terminal growing portions of which are densely filled with protoplasm. Toward the center of the thallus the vacuoles increase in size and may be associated with the presence of metachromatic granules, the latter having nothing in common with bacterial endospores or "micrococci," for which they were mistaken by early observers.

2. The vegetative mycelium attains an extent incomparably greater than the branching figures recorded for bacteria of the acid-fast group, and the hyphae lack the uniformity in diameter generally characteristic of the Schizomycetes.

3. The aërial mycelium produced on suitable substrata by most species occurs usually in the form of a mat of discrete fructifications; but in other species these fructifications are frequently combined to form numerous and peculiar erect Isarioid sporodochia.

4. In any case each individual fructification represents a well characterized sporogenous apparatus, consisting of a sterile axial filament bearing branches in an open racemose or dense capitate arrangement. The primary branches may function directly as

sporogenous hyphae, or may proliferate branches of the second and of higher orders, sporogenesis in the latter case being confined to the terminal elements, the hyphal portions below the points of attachment of branches remaining sterile.

5. Two tendencies in the development of fructifications are recognizable: one leading to an erect drendroidal type, in which successively proliferated fertile elements undergo processes of sporogenesis in continuous sequence; and the other leading to a prostrate racemose type, in which sporogenesis is delayed in the older branches until the younger branches have also attained their final extension. The majority of species show these tendencies combined in different ways.

6. The sporogenous hyphae of most species are coiled in peculiar spirals, sometimes resembling the spores of the hyphomycetous genus *Helicoön*. These spirals exhibit pronounced specific characteristics in the number, diameter, and obliquity of their turns, and especially in the direction of rotation (whether dextrorse or sinistrorse).

7. Sporogenesis, where it can be followed, begins at the tips of the fertile branches and proceeds basipetally. In the larger number of species the process involves the insertion of septa which, in certain cases, are relatively very massive, and in others so thin as to be barely discernible. The disposition of these septa, while the delimited spores undergo maturation processes, varies with the species: (1) they may remain more or less unaltered; (2) they may suffer a median split, the two resulting halves being then separated as the result of the longitudinal contraction of the young spores, leaving alternate portions of hyphal walls completely evacuated; or (3) they may first become considerably constricted and subsequently converted into non-stainable isthmuses connecting the mature spores. The apparent absence of septa in the sporogenous hyphae of other forms is perhaps attributable to optical difficulties.

8. Granules are readily differentiated in the spores of many species which possess the staining properties and uniformity of size characteristic of nuclei; they generally occur singly, but in the larger spores of a few forms two are often found occupying diagonally opposite positions.

9. As in the vegetative thallus, metachromatic granules occur in the aërial mycelium, being very rarely found in the spores or sporogenous hyphae, but becoming very abundant in degenerate sterile hyphae.

10. The older axial filaments of some species show marked distensions which, in extreme cases, result in figures simulating *Leptomitus*. These arise as local distensions at the points of attachment of the more extensive lateral sporogenous processes. Cuneate modifications of the sterile axial filaments below the origins of branches also occur.

11. Curious spherical structures appear regularly in some forms, both in the sterile axial hyphae, where they may contain either a median septum or a number of peripheral metachromatic granules, and in the sporogenous hyphae, where they are associated with the regularly spaced septa.

12. The spores germinate readily in suitable solutions, producing 1–4 germ tubes, the approximate number being more or less characteristic of the species.

13. Owing to the absence of any well defined bacterial characteristics, the writer is of the opinion that the view that *Actinomyces* represents a transition between the Hyphomycetes and the Schizomycetes, as well as the phylogenetic corollary based upon it, may safely be abandoned. If mere size is to be regarded as important, it would appear to be equally profitable to look for bacterial affinities in some ascomycetous and sphaeropsideaceous forms, the hyphae of which are similarly very minute. It is doubtful whether far-reaching taxonomic generalizations can be based on the "acid-fast" staining reaction, especially as this reaction has not played a very important rôle in mycological research. There seems to be no adequate reason why the genus should not be classed in an unqualified manner with the Hyphomycetes, as a mucedineous group with tendencies toward an erect Isarioid habit.

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CRYPTOGAMIC LABORATORIES
HARVARD UNIVERSITY
CAMBRIDGE, MASS.

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EXPLANATION OF PLATES II–IX

All except figs. 7, 8, 70, and 101, which are semidiagrammatical representations with a magnification of approximately 8000, were drawn with the aid of a camera lucida with a magnification of 2750.

PLATE II

Actinomyces I

FIG. 1.—Moderately well developed fructification.

FIG. 2.—Somewhat smaller fructification showing successive order of development: *a*, chain of spores, largely disrupted, developed from termination of axial hypha; *b*, *c*, *d*, secondary branches that have given rise respectively to series of elements *b1–b4*, *c1–c5*, and *d1–d3*.

FIG. 3.—Spore germinating with 4 germ tubes.

Actinomyces II

FIGS. 4–6.—Portions of aërial mycelium, showing conspicuous septa in fertile branches, and relation of latter to axial filaments: *a*, *b*, *c*, branches proliferated successively from same filament; *b1–b3*, *c1–c3*, elements proliferated successively from branches *b* and *c* respectively.

FIG. 7.—Portion of branch *c* (fig. 5) showing attachment of successively formed spiral elements.

FIG. 8*a–f*.—Successive stages in development of fertile hypha.

Actinomyces III

FIGS. 9–14.—Portions of aërial mycelium.

FIG. 15.—Portion of degenerate hypha containing abundance of metachromatic material.

PLATE III

Actinomyces IV

FIG. 16.—Short chain of spores showing nuclei, and 2 deep staining remnants of constricted septa in hyaline isthmuses between spores.

FIGS. 17–20.—Portions of aërial mycelium.

FIG. 28.—Spore germinating with 2 germ tubes.

Actinomyces V

FIG. 21.—Aërial hypha with spiral termination and 2 fertile branches, more mature elements showing failure of spore to develop in proximal portion.

FIG. 22.—Aërial hypha with 2 spiral elements.

FIG. 23.—Young spiral branch of 15 turns attached to axial hypha containing metachromatic granules.

FIGS. 24–26.—Spiral branches soon after insertion of septa, showing cross-walls absent from portion above basal septum.

FIG. 27.—Young spiral branch.

FIGS. 29, 30.—Spiral branches with spores mature, and non-septate portion completely evacuated.

FIGS. 31, 32.—Degenerate filaments containing much metachromatic material.

Actinomyces VI

FIG. 33.—Portion of aërial mycelium showing 2 spiral elements with nuclei in mature spores of one; septum in axial filament associated with basal septum in branch.

FIG. 34.—Similar to fig. 33, but without visible nuclei.

FIGS. 35–37.—Other portions of aërial mycelium.

Actinomyces VII

FIGS. 38–40.—Portions of aërial mycelium with sporogenous branches in various stages of development.

PLATE IV

Actinomyces VIII

FIG. 41.—Sporogenous apparatus with mature spores cohering in zoogloea-like masses.

FIG. 42.—Prostrate hypha containing numerous metachromatic granules and bearing a branch with many crowded spiral ramifications.

FIG. 43.—More open type of sporogenous apparatus with lateral elements attached to axial hypha at intervals.

FIG. 44.—Young sporogenous apparatus with spiral branches more or less crowded.

FIG. 45.—Somewhat older system of spiral hyphae, some of which have become converted into zoogloea-like masses of spores.

FIG. 46.—Lateral element bearing 8 spiral branches.

FIGS. 47, 48.—Portions of degenerate mycelium showing *Leptomitus*-like enlargements occupied by vacuoles, and metachromatic granules in constrictions.

FIGS. 49, 50.—Spores germinating with 1 germ tube.

Actinomyces IX

FIG. 51.—Portion of aërial mycelium showing spiral termination converted into chain of uninucleated spores, and presence of remnants of septa in hyaline isthmuses.

FIG. 52.—Similar to fig. 51, but without indications of septa between spores.

FIG. 53.—Portion of aërial mycelium showing septa in axial filament above insertions of some sporogenous branches.

FIG. 54.—Sporogenous branches with portion below second turn of spiral conspicuously thickened.

FIG. 55.—Sporogenous branch of 11 turns.

FIG. 56.—Spore germinating with 1 germ tube.

FIG. 57.—Sporogenous branch of 15 close spiral turns.

Actinomyces X

FIGS. 58–60.—Portions of aërial mycelium.

FIGS. 61, 62.—Spores germinating with 1 and 2 germ tubes respectively.

PLATE V

Actinomyces XI

FIGS. 63, 64.—More or less erect fructifications terminating long prostrate filaments, showing origin of groups of sporogenous branches from local hyphal distensions occupied by conspicuous vacuole.

FIGS. 65, 66.—Intermediate portions of aërial mycelium.

FIG. 113.—Spore germinating with 1 germ tube.

Actinomyces XII

FIGS. 67, 68.—Erect fructifications terminating long prostrate aërial filaments, exhibiting a pronounced tendency toward successive type of development, and showing cuneate hyphal enlargements below insertions of branches.

FIG. 69.—Intermediate portion of aërial mycelium.

FIG. 70*a–e*.—Progressive stages in development of sporogenous hypha, occurring in this and numerous other species.

FIG. 71.—Spore germinating with 3 germ tubes.

PLATE VI

Actinomyces XIII

FIG. 72.—Two portions, *a* terminal, *b* subterminal, of one long, unbranched, continuous sporogenous hypha showing very slight spiral tendency.

FIG. 73.—Chain of spores with deep staining polar granules.

FIGS. 74, 75.—Portions of aërial mycelium showing branching.

Actinomyces XIV

FIGS. 76–81.—Portions of aërial mycelium showing arrangement of sporogenous branches on hyphae, and method of sporulation.

PLATE VII

Actinomyces XV

FIG. 82.—Portion of aërial mycelium: elements *a1–a3* and *b1–b3* successively proliferated from branches *a* and *b* respectively.

FIG. 83.—Sporogenous branch *a* with 2 secondary branches; younger (*a3*) associated with a septum above insertion (successive type); older (*a2*) not set off by septum.

FIG. 84.—Axial filament with 3 branches, bearing successively poliferated elements *a1–a3*, as well as branch *ax*, latter not associated with septum in primary branch above point of attachment.

FIG. 85.—Fructification developed entirely in successive sequence, with 2 chains of uninucleated spores.

FIGS. 86–88.—Spores germinating with 2 germ tubes.

Actinomyces XVI

FIG. 89.—Large fructification consisting of axial filament *a–a1*, with whorl of 5 primary branches *a2–a6*, each bearing 1 or more secondary branches.

FIG. 90.—Spiral termination of sporogenous branch.

FIG. 91.—Old filament containing many metachromatic granules.

PLATE VIII

Actinomyces XVII

FIG. 92.—Portion of aërial mycelium showing spherical structures associated with septa in local distensions of sporogenous branch.

FIGS. 93, 94.—Portions of aërial mycelium, some lateral elements bearing secondary branches (indicated by numerals above 1) developed successively; 94*c*, unusually long fertile branch.

FIG. 95.—Portion of aërial mycelium similar to one shown in fig. 92.

FIGS. 96–100.—Spores germinating with 1 or 2 germ tubes.

FIG. 101*a–e*.—Successive stages in development of sporogenous branch, *cx* and *cy* representing either alternative or probably successive stages.

PLATE IX

Actinomyces XVIII

FIG. 102.—Sporogenous branch of usual type soon after appearance of septa.

FIG. 103.—Portion of fructification bearing aberrant fertile branches without spiral terminations.

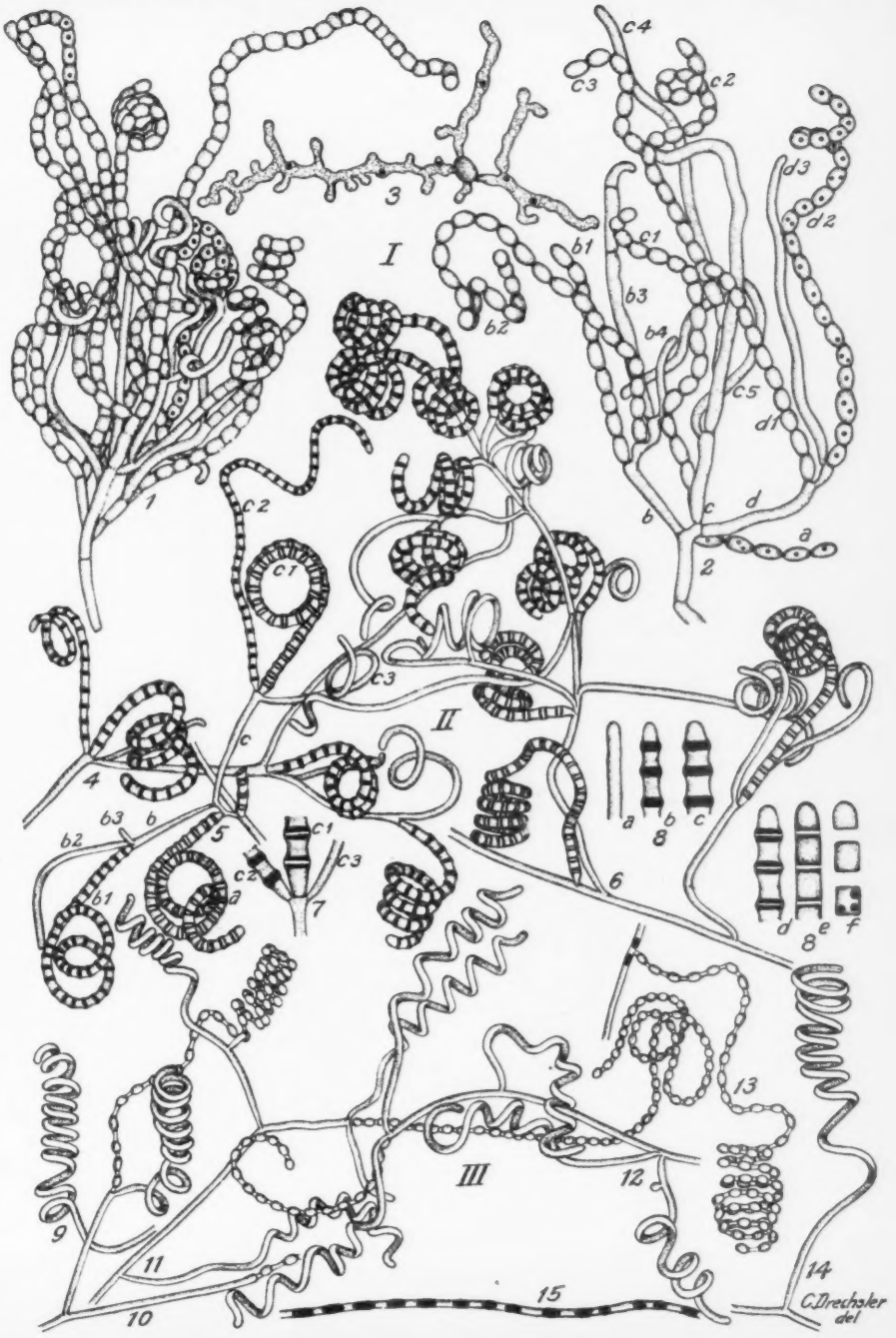
FIG. 104.—Aërial filament with several spiral branches borne terminally.

FIG. 105.—Chain of mature spores developed from branch of spiral type.

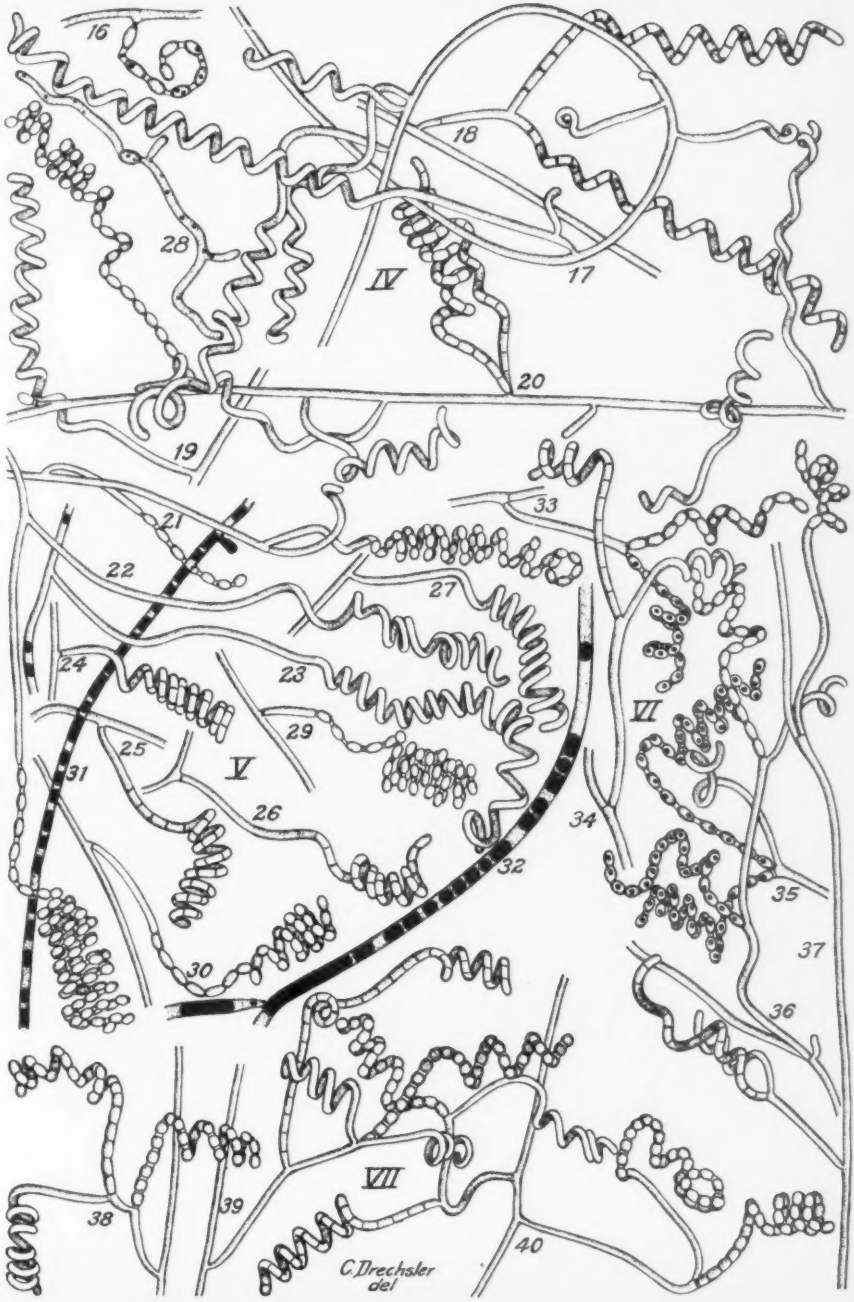
FIG. 106.—Degenerate axial filament containing large vacuoles and spherical structures and bearing a fertile spiral branch.

FIGS. 107, 108.—Portions of aërial mycelium showing fertile hyphae of spiral type.

FIGS. 109–112.—Spores germinating with 1 or 2 germ tubes.



DRECHSLER on ACTINOMYCES



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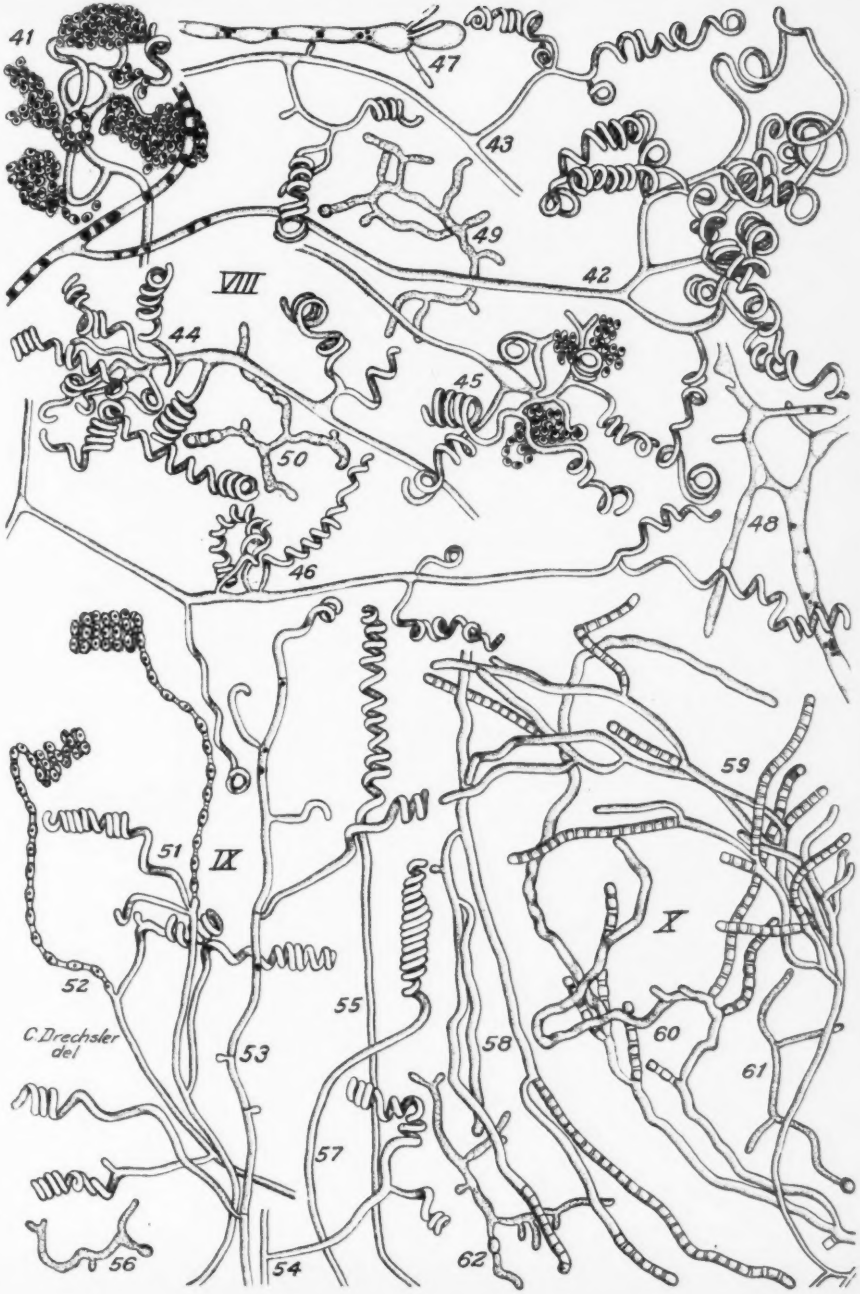

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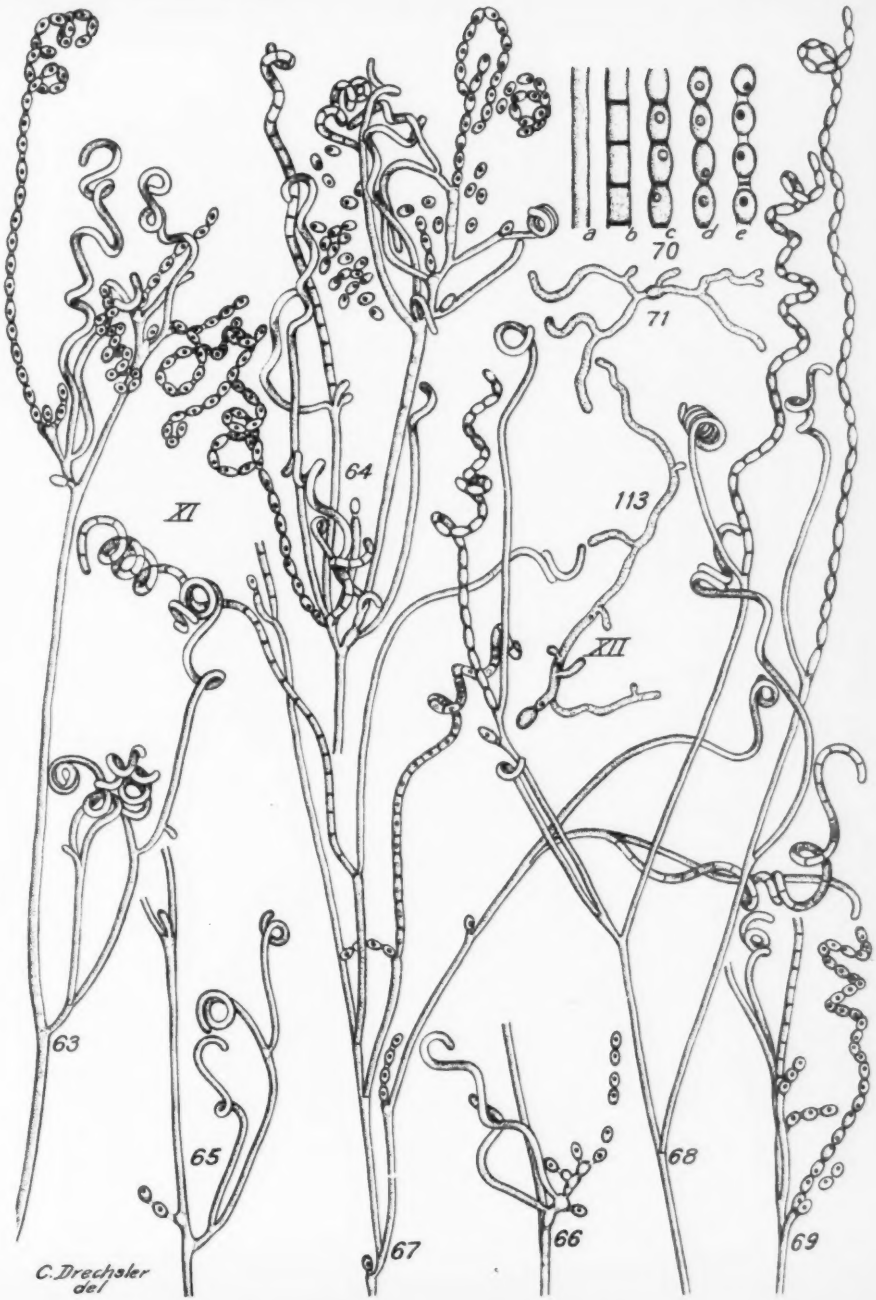

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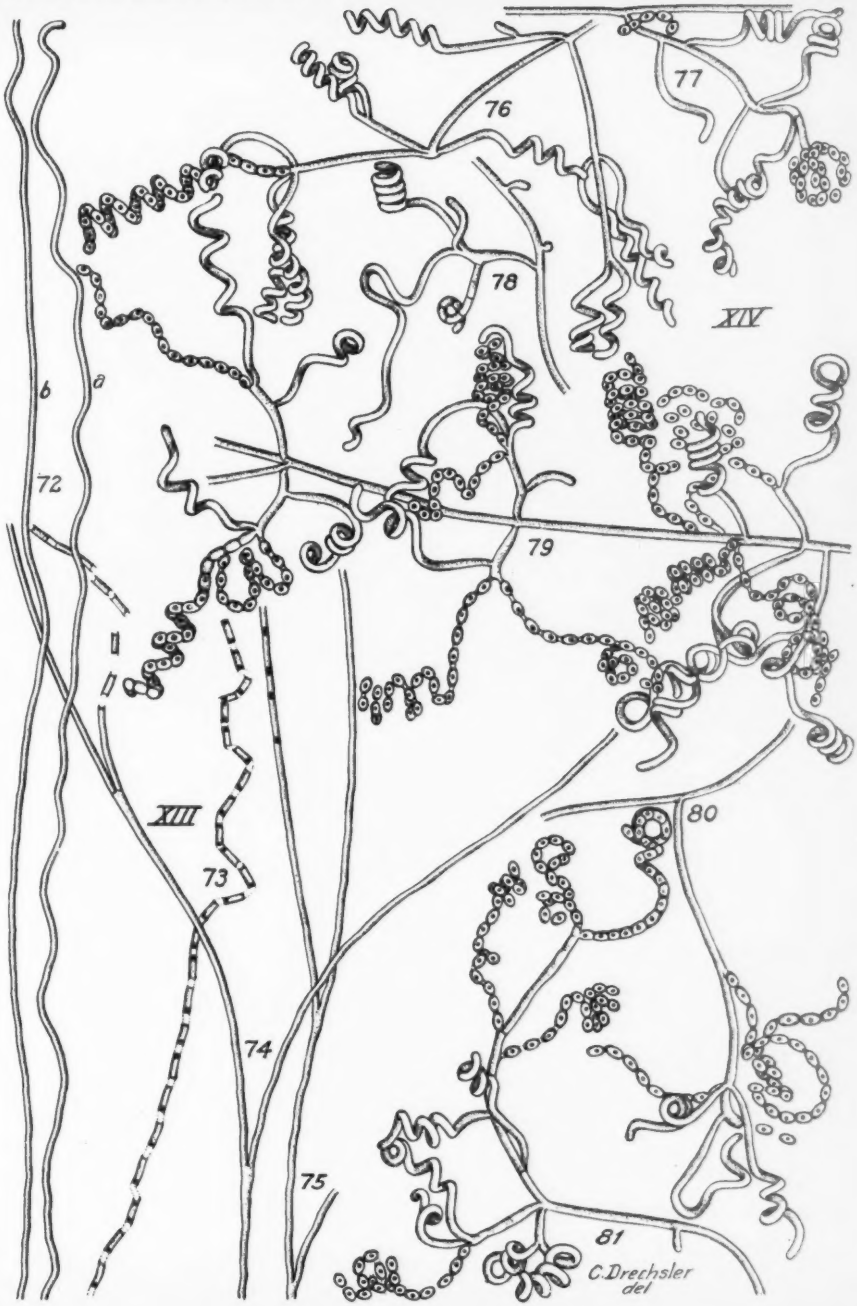

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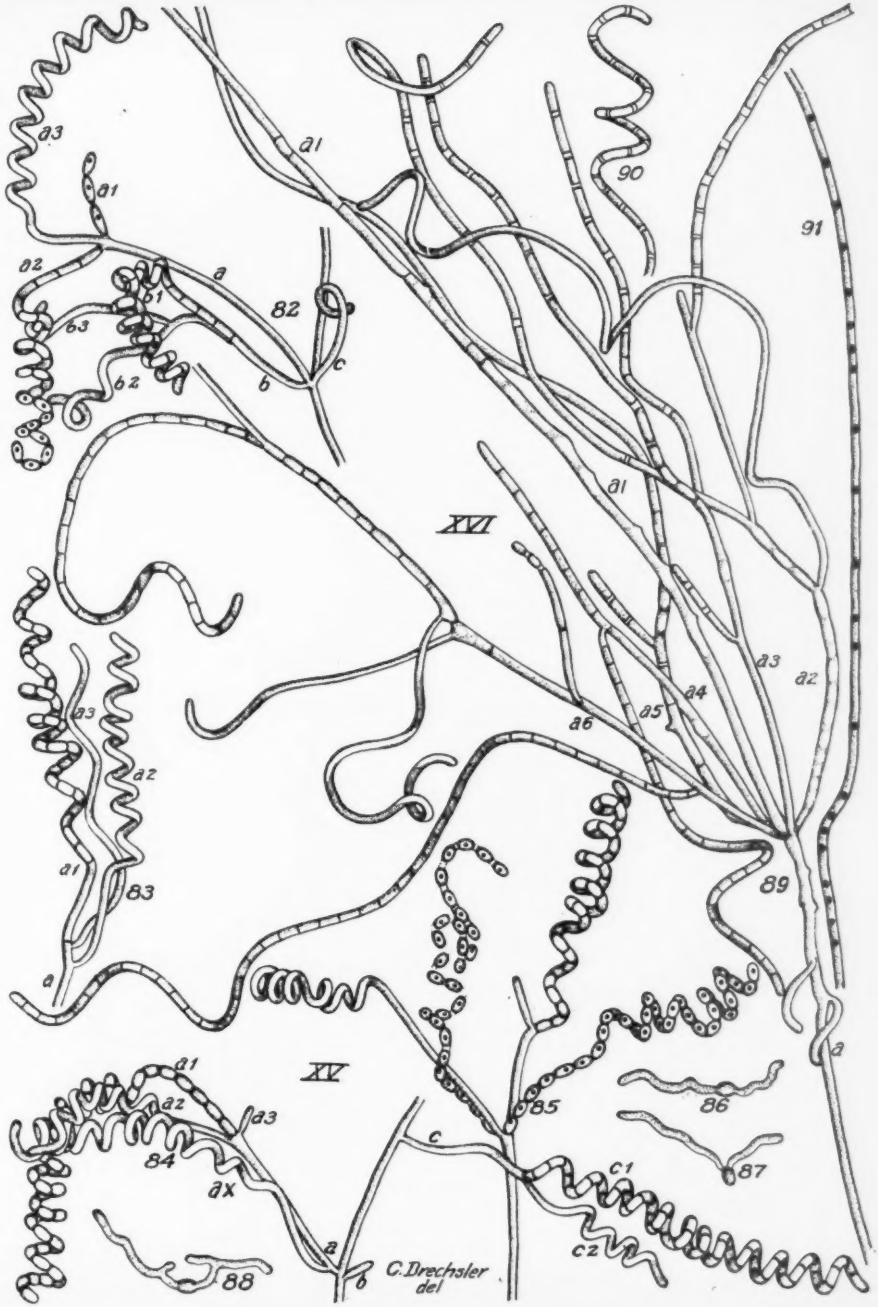

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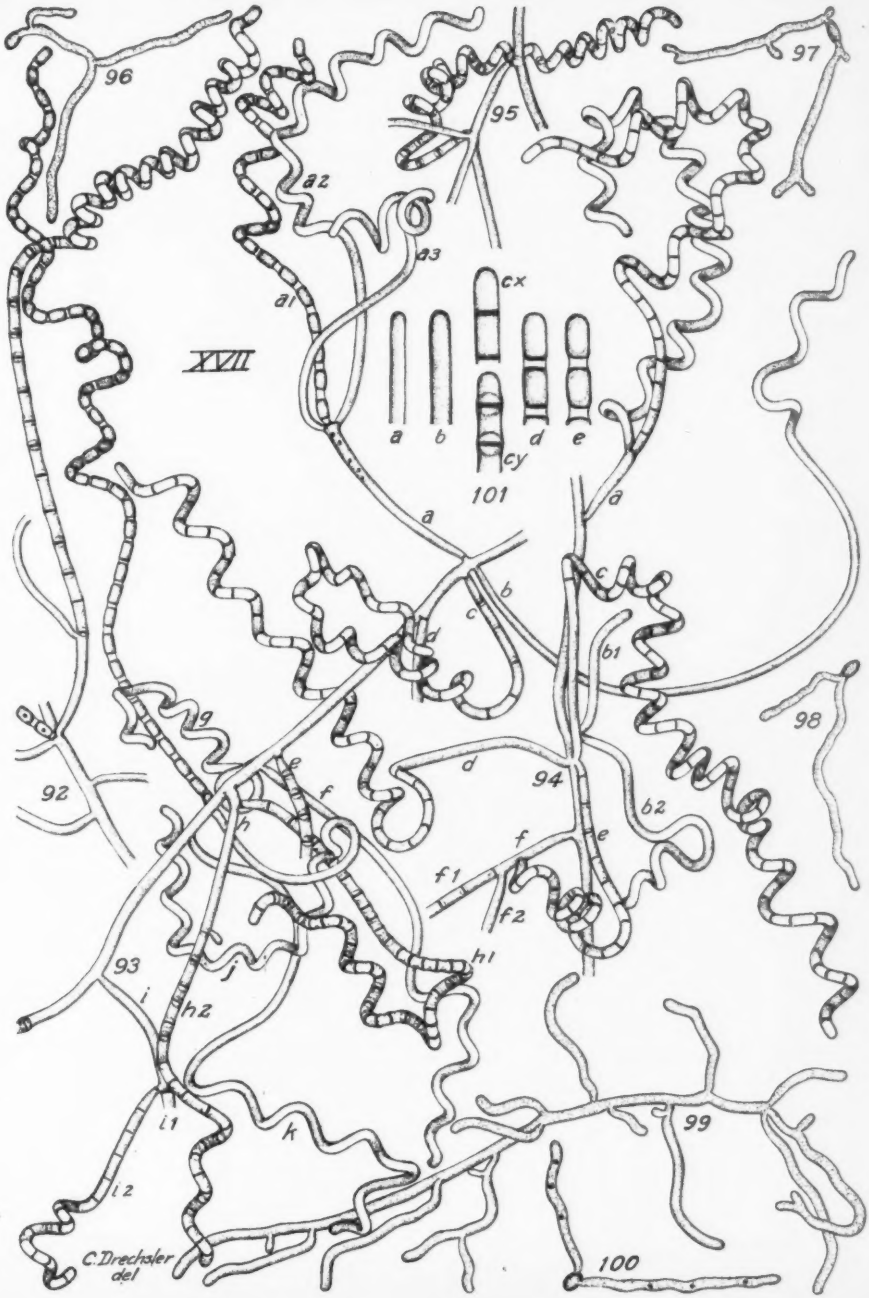

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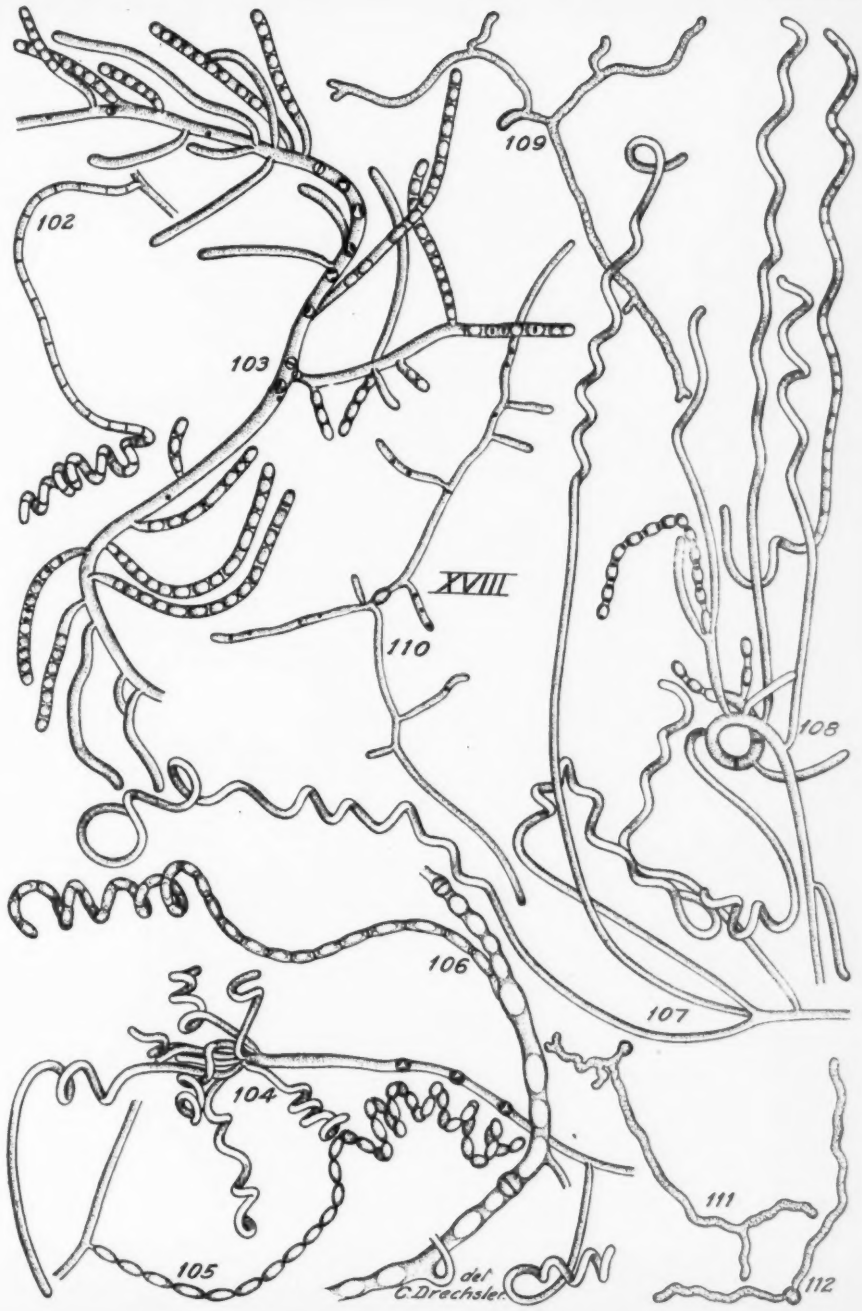

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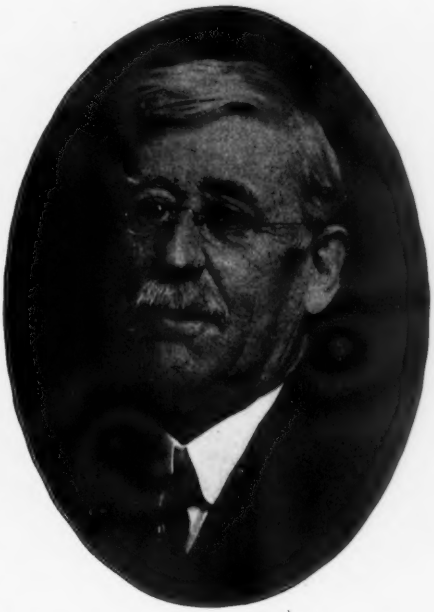
BRIEFER ARTICLES

BYRON DAVID HALSTED

(WITH PORTRAIT)

With the passing of BYRON DAVID HALSTED we have lost another of our pioneer botanists. Although not one of the earliest pioneers, he was a pioneer in many respects. He was one of that group of botanists who laid the foundation of the science in America at a time when the subject was recognized by very few American colleges and universities. He was one of that still smaller group who took up the study of applied botany and worked faithfully for its advancement.



Our younger plant pathologists know how difficult it is to find a disease of an economic crop that is not at least mentioned in his reports. He was among the first to report the introduction of several foreign pathogenic organisms.

Born at Venice, Cayuga County, New York, June 7, 1852, he was left an orphan at an early age and was cared for by relatives. He graduated from the Michigan Agricultural College with the degree of B.S. in 1871, and received the M.S. degree from the same college in 1874. In 1878 he received the Sc.D. degree from Harvard, being the first man to take the doctorate in cryptogamic botany from that university. He was managing editor of the *American Agriculturist* from 1880 to 1885; Professor of Botany in the Iowa Agricultural College 1885–1889; and Professor of Botany in Rutgers College

and Botanist of the New Jersey Agricultural Experiment Station from 1889 until his death, August 26, 1918. Had he lived until February, 1919, he would have rounded out a full 30 years in the service of the state of New Jersey. During the greater part of these 30 years he was active in both College and Station, but in the latter part of his career poor health necessitated his retirement from the classroom.

Although a very busy man, he found time to serve his science by acting as Associate Editor of the *Bulletin of the Torrey Botanical Club* from 1890 to 1893 and as a contributor to the *Systematic Flora of North America*. In 1877 the Massachusetts Horticultural Society honored him with its silver medal. He was a member of a number of scientific societies, serving as president of the Society for the Promotion of Agriculture from 1877 to 1879 and of the Botanical Society of America in 1900–1901.

Dr. HALSTED was a true lover of nature, and nature made him a most warm hearted and lovable man. He loved to commune with nature and was a most enthusiastic collector. In addition to his own studies, he furnished a great quantity of material for study by other mycologists and from which many new species were described; in fact, the mycological collections not only of America but of the entire world contain material collected by him.

The writer looked upon him as a botanist of the old school, and yet he was an up-to-date botanist in every way. After devoting the greater part of his career to mycology, poor health and a failing eyesight forced him to abandon his favorite line of work. He could not leave the field of botany, however, but merely transferred his efforts to a line of plant breeding which did not require the use of the microscope, and worked with the renewed energy and the enthusiasm of a boy.

Dr. HALSTED was more than a botanist; he was a broad, scholarly man and a public spirited citizen. He was always interested in athletics and in his youth was an amateur baseball pitcher. He never lost his interest in the sport, but was a regular attendant at intercollegiate games, always placing himself so that he could observe the work of the pitcher. His love for literature and his keen interest in the state and community were made manifest by a poem which he wrote on the occasion of a civic parade when the nation was called to arms in 1917.

He was the author of many papers, and while most of us will think of him as a scientist, it should be remembered that many of his papers had to do with other subjects.—MEL. T. COOK, *New Jersey Agricultural Experiment Station, New Brunswick, N.J.*

CURRENT LITERATURE

BOOK REVIEWS

Soil conditions and plant growth

The rapid progress that is being made in the scientific field encompassed by plant physiology and soil science is evidenced in the appearance of a third and enlarged edition of RUSSELL'S[1] *Soil conditions and plant growth* in the series of Monographs on Biochemistry edited by PLIMMER and HOPKINS. The first edition (1912) contained 166 pages, including 13 pages of citations; the second edition (1915) contained 188 pages, including 14 pages of citations; and the third edition (1917) contains 240 pages, including 18 pages of citations. The growth of the subject is marked by the addition of a chapter on "The relationship between the micro-organic population of the soil and the growth of plants" to the second edition, and a chapter on "The colloidal properties of soil" to the third edition. In his preface to this new edition the author states that he has not attempted to refer to every paper published on the subject since the first edition, but that his "guiding principle has been to include only those that brought in some new idea or profoundly modified an old one." The choice of papers is naturally a question of judgment, yet the reviewer feels that certain important omissions have been made, and he will therefore direct the reader's attention to these in the hope that they may serve to supplement this admirable treatment of practical plant physiology. Certain papers published since 1917, and therefore not referable to as "omissions," will be included in order to bring the subject up to date.

Chapter II on "The requirements of plants" presents a modern physiological basis for the rest of the book. This chapter has been enlarged to the extent of 5 pages over the first edition. In the discussion of limiting factors the treatment of oxygen (only 9 lines) and temperature seems quite inadequate. Attention is therefore directed to the following papers:

On temperature relations of plants.—LEITCH, I., Some experiments on the influence of temperature on the rate of growth of *Pisum sativum*. Ann. Botany **30**:25–46. 1916; LEHENBAUER, P. A., Growth of maize seedlings in relation to temperature. Physiol. Researches **1**:247–288. 1914; LEPESCHKIN, W. W., Zur Erkenntnis der Einwirkung supramaximaler Temperaturen auf die Pflanze. Ber. Deutsch. Bot. Gesell. **30**:703–714. 1912; MAXIMOW (MAKSIMOV), N. A., Chemische Schutzmittel der Pflanzen gegen Erfrieren. Ber. Deutsch. Bot.

[1] RUSSELL, E. J., Soil conditions and plant growth. 3d ed. pp. 243. *figs.* 14. New York: Longmans, Green and Co. 1917.

Gesell. **30**:52–65, 293–305, 504–816. 1912; GROVES, J. F., Temperature and life duration of seeds. BOT. GAZ. **63**:169–189. 1917.

On oxygen relations of plants.—SHULL, CHARLES A., The oxygen minimum and the germination of *Xanthium* seeds. BOT. GAZ. **52**:453–477. 1911; CANNON, W. A., and FREE E. E., The ecological significance of soil aëration. Science N.S. **45**:178–180. 1917; LIVINGSTON, B. E., and FREE, E. E., The effects of deficient soil oxygen on the roots of higher plants. Article in "Contributions to Plant Physiology." Johns Hopkins University. Reprint from Johns Hopkins University Circular. March 1917.

The new edition deserves high praise for its comprehensive treatment and impartial judgment on the modern developments in soil chemistry. The discussion on the use of dilute acids in soil analysis, based on the author's own work in the Rothamsted laboratories, is the first contribution on this subject that has forsaken empirical experimental methods and adopted a modern physico-chemical procedure. The review of the highly controversial literature on soil acidity is eminently fair. The attention of the reader is called to the following recent papers, each of which contains an extensive bibliography: CHRISTENSEN, H. R., Experiments in methods for determining the reaction of soils. Soil Science **4**:115–178. 1917; TRUOG, E., Soil acidity. I. Its relation to the growth of plants. Soil Science **5**:169–195. 1918; RICE, F. E., and OSUGI, S., The inversion of cane sugar by soils and allied substances and the nature of soil acidity. Soil Science **5**:333–358. 1918.

The biological aspects of soil science are fully treated, including the author's own interesting ideas on soil protozoa and partial sterilization. The reviewer feels that RUSSELL has been somewhat partial to his own views in not referring to SHERMAN'S studies. SHERMAN concludes, on the basis of his experiments, that "no evidence has been obtained which indicates that the beneficial effect of partial sterilization is due to the elimination of a biological factor which is harmful to the bacteria." BOLLEY'S interesting views on "Soil sanitation" deserve mention in a chapter that bears such an all-inclusive title as "The relationship between the micro-organic population of the soil and the growth of plants." The following papers should be read in connection with the chapter: SHERMAN, J. M., Studies on soil protozoa and their relation to the bacterial flora. Jour. Bacteriology **1**:35–66, 165–185. 1916; KOPELOFF, N., and COLEMAN, D. A., A review of investigations in soil protozoa and soil sterilization. Soil Science **3**:197–269. 1917; BOLLEY, H. L., Wheat-soil troubles, causes of soil sickness, etc. Bull. 107. N.D. Agric. Exper. Sta. 1913; BOLLEY, H. L., Conservation of the purity of soils in cereal cropping. Science N.S. **32**:529–541. 1910; BOLLEY, H. L., Cereal cropping: sanitation, a new basis for crop rotation, manuring, tillage, and seed selection. Science N.S. **38**:249–259. 1913; HOPKINS, C. G., The bread supply. Science N.S. **38**:479–481. 1913.

KEEN'S mathematical studies on the retention of water by soil are amply discussed, but no mention is made of SHULL'S even more important contribution to the problem of the wilting coefficient. The reader is therefore referred to the

following: SHULL, C. A., Measurement of the surface forces in soils. BOT. GAZ. 62:1–31. 1916.

The final chapter is devoted to a theoretical discussion of soil analysis. An appendix describes analytical methods used in England. The reviewer believes that the reader should be cognizant of the following discussions of American methods: BEAR, F. E., and SALTER, R. M., Methods in soil analysis (Technical Bulletin). Bull. 159. West Virginia Agric. Exper. Sta. Morgantown. 1916; AMES, J. W., and SCHOLLENBERGER, C. J., Liming and lime requirement of soil. Bull. 306. Ohio Agric. Exper. Sta. Wooster. 1916; TRUOG, E., A new test for soil acidity. Bull. 249. Wisconsin Agric. Exper. Sta. Madison. 1915; BOUYOUCOS, GEO. J., and MCCOOL, M. M., The freezing point method as a new means of measuring the concentration of the soil solution directly in the soil. Tech. Bull. 24. Michigan Agric. Exper. Sta. East Lansing. 1915.—H. L. WALSTER.

MINOR NOTICES

History of phytopathology.—WHETZEL,[2] in his *History of phytopathology*, aims "only to set forth in outline what appear to be the most outstanding features in the evolution of the science, and to indicate the proper relation thereto of the men who have chiefly shaped its progress." The chief captions are: (1) The Ancient Era, to the end of the 5th century (5 pp.); (2) The Dark Era, 6th to 16th centuries (1 p.); (3) The Premodern Era, 1600 to about 1850 (19 pp.); (4) The Modern Era, 1853 to about 1906 (65 pp.); (5) The Present Era, 1906 (8 pp.). As is indicated by the page allotment, the first and second topics are treated very briefly, being barely sketched. The third and fifth topics are treated somewhat more fully, while the most page space is given to "The Modern Era." The book is in the main a series of brief biographical sketches, often with portraits, arranged chronologically under the captions indicated. It will be a convenient reference book for those who may need ready access to such biographies.—F. L. STEVENS.

Winter botany.—To supplement his pocket manual of woody plants, already noted in this journal,[3] TRELEASE[4] has compiled and published a companion volume for use in naming our common trees and shrubs when without foliage. The range, extending to 326 genera and over 1000 species, includes most introduced as well as native woody plants. The notable features of the volume, aside from its convenient pocket size and abundant illustrations from most accurate drawings, are the numerous keys and the many citations of literature dealing with winter characters of the various genera and species. The

[2] WHETZEL, H. H., An outline of the history of phytopathology. pp. 130. Saunders Co. 1918.

[3] BOT. GAZ. 65:194. 1918.

[4] TRELEASE, WILLIAM, Winter botany. 16mo. pp. 394. *figs.* 327. Urbana, Ill. Published by the author. 1918. $2.50.

drawings are of bud, leaf-scar, pith, and other twig characters upon which the keys are based, so that with the use of a hand lens it should be possible to determine readily the genera, and for the most part the species, for native and introduced trees and shrubs. The author is to be congratulated in making such a fund of unusual information available in such a compact and readily available form.—GEO. D. FULLER.

American trees.—Another book on trees, by EMERSON and WEED,[5] has been added to the already large number upon the same subject. It is essentially a book for the amateur, since its chief virtue lies in the excellent photographs by EMERSON, an entire page being devoted to each species. The absence of keys of any sort renders the book comparatively useless for the identification of an unknown species, but the quality and abundance of the illustrations will make it one the tree lover will wish to have upon his table.—GEO. D. FULLER.

NOTES FOR STUDENTS

Physiological balance in soil and other nutrient solutions.—HIBBARD[6] has just published a piece of work on physiological balance in soil solution which is to mark a decided advance (both theoretically and practically), if the future development of the work approximates its present promise. He extracted the soil solution from an infertile very sandy soil and from a fertile sandy loam by the Van Suchtelen oil pressure method as improved and extended in usefulness by MORGAN.[7] HIBBARD speaks of this as giving a more concentrated solution than any other extraction method. The solution thus extracted from the poor sandy soil had an osmotic pressure of 0.193 atmospheres, and that from the good soil 1.81 atmospheres. The soil extracts showed an order of production similar to the soils from which they came.

The soil extracts were used instead of distilled water to prepare the Shive 3-salt (KH_2PO_4, $Ca(NO_3)_2$, $MgSO_4$) nutrient solution. The total concentration of nutrient salts added gave an osmotic pressure of 1.75 atmospheres, and in the 36 different solutions made up from each soil extract and from distilled water the proportions of each salt varied from 10 to 80 per cent of the total nutrient salt osmotic concentration.

In the nutrient solution made from the extract of the poor soil the optimum osmotic proportions of the KH_2PO_4, $Ca(NO_3)_2$, and $MgSO_4$ for the growth of Fultz wheat were 7:1:2 respectively, with a total osmotic pressure of (1.75+0.193) 1.94 atmospheres; in that made from the extract of the good soil 2:7:1 respectively, with a total osmotic pressure of (1.75+1.81) 3.56 atmospheres; and in that made with distilled water 5:2:3, with a total osmotic pressure of

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[5] EMERSON, ARTHUR I., and WEED, C. M., Our trees, how to know them. New ed. pp. xxi+295. *pls. 149*. Philadelphia: Lippincott Co. $3.50.

[6] HIBBARD, R. P., Physiological balance in the soil solution. Tech. Bull. Mich. Agric. Exper. Sta. no. 40. pp. 44. 1917.

[7] Tech. Bull. Mich. Agric. Coll. Exp. Sta. no. 28.

1.75 atmospheres. Analyses of the soil extract of the poor soil showed it to be proportionally low in K_2O and P_2O_5. Also addition of KH_2PO_4 to the poor soil at the rate of 150–300 pounds to the acre greatly increases the growth of wheat. A similar analysis of the soil extract of the good soil showed it to be as poor in nitrates, nitrites, and ammonium salts as the extract from the poor soil, but with a relatively high calcium magnesium ratio. Addition of $Ca(NO_3)_2$ to the good soil at the rate of 200–400 pounds to the acre greatly improved the growth of the plants. HIBBARD concludes that the soil is deficient in calcium and perhaps nitrates. If, as HIBBARD's results seem to indicate, one can tell the fertilizer needs of a soil by such a study of the soil extracts, he has given us a new practical method of great significance. He has also hinged the fertilizer question largely on the balance between nutrient ions.

HIBBARD worked only on the early stage of the growth of wheat. It would be of interest to know whether the same ratios apply to the middle and late stages. It will also be interesting to see the nature of these ratios when plants with high sulphur demands (alfalfa, cabbage, etc.), those with high potash demands (potato, tobacco, etc.), or those with high general nutrient demands (squash, cucumber, etc.) are used. These stand in contrast with the wheat with its moderate demands. The method also needs the test of a great range of soil types of various degrees of fertility. Considering all of these variables, the application of the method may become rather complex. The work is sure to stimulate a great amount of investigation.

The use of a 3-salt nutrient solution requires that the variation in concentration of the several nutrients shall always be in pairs. In Shive's solution a change in concentration of sulphur is always accompanied by a corresponding change in the concentration of magnesium, potassium by phosphorus, and calcium by nitrogen. There is no reason for thinking that the magnesium demands of a plant are thus tied up with the sulphur demands; in fact, in soils we think of the sulphur as existing mainly as calcium sulphate, and in fertilizer practice it is more generally added in this form. This is not an adverse criticism of the use of 3-salt nutrient solutions; it is rather an acknowledgment of the complexity and innate difficulties of the problem. Since so many variations in proportions of nutrients are possible, the investigator must study only a portion of these, if he completes his investigations within a reasonable time.

LIVINGSTON and TOTTINGHAM[8] recognize this shortcoming of the 3-salt nutrient solution when they mention the 6 possible combinations of salts and proceed to investigate the best proportions in the second combination. The possible combinations are:

I	II	III	IV	V	VI
$Ca(NO_3)_2$	$Ca(NO_3)_2$	$Ca(H_2PO_4)_2$	$Ca(H_2PO_4)_2$	$CaSO_4$	$CaSO_4$
KH_2PO_4	K_2SO_4	KNO_3	K_2SO_4	KNO_3	KH_2PO_4
$MgSO_4$	$Mg(H_2PO_4)_2$	$MgSO_4$	$Mg(NO_3)_2$	$Mg(H_2PO_4)_2$	$Mg(NO_3)_2$

[8] LIVINGSTON, B. E., and TOTTINGHAM, W. R., A new 3-salt nutrient solution for plant cultures. Amer. Jour. Bot. 5:337–346. 1918.

They grew Fulcaster wheat for 18 days in 12 different molecular proportions of the salt combination II. The nutrient solutions had a total osmotic pressure of 1.75 atmospheres. Of the 12 volume molecular proportions tried the following proved best: KNO_3 0.0216, $CaH_2(PO_4)_2$ 0.0026, $MgSO_4$ 0.0150. This finding must not be taken too seriously, however, for it is based on the extremely slim experimental evidence of a single set of cultures with 6 seedlings in each concentration; also very different molecular proportions (KNO_3 0.0036, $CaH_2(PO_4)_2$ 0.0078, $MgSO_4$ 0.0300) gave almost as good results as this optimum and very much better results than the intermediate combinations.

Using combination I (Shive's solution) with a total osmotic pressure of 1.75 atmospheres, SHIVE and MARTIN[9] have determined the optimum volume molecular proportions for the development of buckwheat during its early period of growth (24 days) and during its period of maturity. The following table gives the results based on maximum production of dry weight:

			VOLUME-MOLECULAR PARTIAL CONCENTRATION			YIELD OF TOPS, ROOTS, SEEDS RELATIVE TO THOSE FROM	
			KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Knop's solution taken as 1.00	Tottingham's solution taken as 1.00
Buckwheat grown to flowering (early growth period)	Water cultures	Tops	0.0144	0.0052	0.0200	1.61	1.33
		Roots	0.0144	0.0052	0.0200	1.58	1.27
	Sand cultures	Tops	0.0144	0.0052	0.0200	1.38	1.25
		Roots	0.0180	0.0104	0.0050	1.47	1.16
Buckwheat grown to maturity (late growth period)	Water cultures	Tops	0.0108	0.0130	0.0100	1.40	1.35
		Roots	0.0108	0.0130	0.0100	1.50	1.39
		Seeds	0.0108	0.0078	0.0200	1.28	1.74
	Sand cultures	Tops	0.0108	0.0130	0.0100	1.40	1.26
		Roots	0.0108	0.0130	0.0100	1.27	1.26
		Seeds	0.0108	0.0130	0.0100	1.24	1.17

It is interesting to see SHIVE'S solution far superior to KNOP'S and to TOTTINGHAM'S for growth of buckwheat. In former work by SHIVE the maximum growth of wheat tops appeared in the following molecular proportions: KH_2PO_4, 0.0180; $Ca(NO_3)_2$, 0.0052; $MgSO_4$, 0.0150. The $Ca(NO_3)_2$ needs for buckwheat rises greatly in the late growth period, while the KH_2PO_4 and $MgSO_4$ requirements both fall.

Many of the excellent methods used in these 3-salt nutrient solution studies were worked up by MCCALL. MCCALL and RICHARDS[10] summarize their

[9] SHIVE, J. W., and MARTIN, W. H., A comparison of the salt requirements for young and for mature buckwheat plants in water cultures and sand cultures. Amer. Jour. Bot. 5:186–191. 1918.

[10] MCCALL, A. G., and RICHARDS, P. E., Mineral food requirements of the wheat plant at different stages of its development. Jour. Amer. Soc Agron. 10:127–134. 1917.

results on wheat in the following table, showing the mean molecular proportions of the 3 component salts and the ionic ratios for the culture solutions giving the best and the poorest growth of wheat during the different periods of development. They promise a full discussion of data with the publication of

Series and growth rank		Mean molecular proportions in tenths of total concentration		
		KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
Series 1 first period	Best 9	3.4	4.7	1.9
	Poorest 9	3.8	2.0	4.2
Series 2 second period	Best 9	3.5	4.6	1.9
	Poorest 9	2.7	1.5	5.8
Series 3 third period	Best 9	2.0	5.5	2.5
	Poorest 9	6.1	1.8	2.1

results of experiments, now being carried on at the Maryland Experiment Station on the nutrient requirements of soy beans and buckwheat.

All this work indicates that SACHS, KNOP, and others had not nearly exhausted the subject of desirable nutrient solutions for water and sand cultures, but that we still have much to learn. The concentration of the nutrient salts used in these solutions are far above those existing in the general soil solution, and upon the whole the immediate significance of this work in problems of soil fertility is not evident. In the soil the dissolving power of well developed root hairs in contact with nutrients of low solubility introduces a new and important feature. It must be stated, however, that HIBBARD'S work offers indications of an important bearing of this method on questions of soil fertility.—WM. CROCKER.

Respiration and age of plant organs.—NICOLAS[11] has studied the respiration of very young leaves and leaflike structures in comparison with that of the corresponding fully developed organs taken from older parts of the same plants. In a few cases he included also a comparative study of the respiration of sections of the stem or branch bearing the young and old leaves. His material was selected from 15 different species, including annuals, biennials, and perennials. The studies were conducted by the method of confined atmospheres in absence of light at temperatures from 15 to 24° C., the gas analysis being made with the Bonnier-Mangin apparatus. Respiratory intensities were calculated on the basis of the fresh weight of the materials used. The "internal respiration" also was determined in a few cases, using hydrogen atmospheres. Respiratory quotients CO_2/O_2 and the ratio I/N between "internal respiration" and normal

[11] NICOLAS, G., Contribution a l'étude des variations de la respiration des végétaux avec l'age. Rev. Gen. Botanique **30**: 214–225. 1918.

respiration are given, the former varying from 0.58 to 1.06 and the latter from 0.13 to 1.38.

In every case the young organ, whether leaf, cladode, or branch, had a greater respiratory intensity, a larger respiratory coefficient, and a lower intramolecular respiration than the corresponding older organ. This is true whether very young organs or organs in a somewhat later stage of development are compared with fully developed organs of the same year's growth or fully developed organs of the current year's growth are compared with organs of the previous year's growth (leaves of *Olea europea* L.).

The magnitude of the differences in respiratory intensity between young and old organs varied rather widely in the different species studied, and was evidently related to the relative differences in age. In general the respiratory intensity of the young organs was from 3 to 7 times that of the older organs.

The author reviews the work of previous investigators, all of whom agree that the respiratory intensity of young organs is greater than that of the corresponding older organs. Especially interesting in this connection are the studies of BONNIER and MANGIN,[12] of MAIGE,[13] and of Mme MAIGE,[14] as cited by NICOLAS in his article. BONNIER and MANGIN found two maxima for respiratory intensity in the seasonal development of a plant, one at the opening of the leaf buds or at the germinative period, the other at the time of flowering. MAIGE found that, while the respiratory intensity of flowers decreases with age when calculated on either wet or dry weight, it increases when stated in terms of amount of gaseous exchange per individual flower, and Mme MAIGE pointed out a decrease in respiratory intensity in each organ of the flower except the gynecium, where it sometimes increases with age.

The author gives reasons why it is thought that the respiratory differences observed between young and old organs cannot be explained by the absence of well developed cuticle in very young organs, the relative amounts of chlorophyll in the tissues, or the greater acidity of young organs, and raises the question whether they may not be referred to the activities of diastase and oxidase. Finally, the author refers to a previous paper with Mme MAIGE,[15] in which it was shown that increase in turgescence increases both the respiratory quotient and the respiratory intensity, and concludes that the turgescence of young

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[12] BONNIER, GASTON, and MANGIN, LOUIS, Recherches sur les variations de la respiration avec le développement des plantes. Ann. Sci. Nat. Bot. VII. **2**:315–364. 1885.

[13] MAIGE, M. A., Recherches sur la respiration de la fleur. Rev. Gen. Botanique **19**:1–28. 1907.

[14] MAIGE, Mme G., Recherches sur la respiration des différéntes piéces florales. Ann. Sci. Nat. Bot. IX. **14**:1–62. 1911.

[15] MAIGE, Mme A., and NICOLAS, G., Recherches sur l'influence des variations de la turgescence sur la respiration de la cellule. Rev. Gen. Botanique **22**:409–422. 1910.

growing organs is of importance in determining the character and amount of their respiration.

The author's conclusions are as follows: "In young organs, principally leaves, intramolecular combustions are more complete than in older organs; young tissues consume much more oxygen than those completely developed, fix relatively less, and thus set at liberty greater quantities of energy, which they use in growth."—G. T. HARRINGTON.

Catalase, respiration, and vitamines.—DUTCHER[16] finds that the catalase activity of polyneuritic pigeons is very low, and that it rises to normal when the fowl is fed water soluble vitamine. His results are given in the following table:

CATALASE ACTIVITY OF TISSUES

Tissue	Polyneuritic pigeons, percentage of normal	Polyneuritic pigeons receiving water soluble vitamine, percentage of normal
Liver	44	110
Kidney	53	102
Pancreas	33	115
Heart	34	86
Breast	13	152
Lung	57	84
Blood	75	56
Average	44	101

The author says: "It is probable that polyneuritis is accompanied by incomplete or partial oxidation, with accumulation in the tissues of products of incomplete oxidation. It is also probable that water soluble vitamines function, directly or indirectly, in stimulation of oxidation processes, thereby clearing the tissues of toxic materials. When pigeon tissues are arranged in the order of their catalase content (as measured by the oxygen liberated from hydrogen peroxide), tissues group themselves in the order of their metabolic activity and also in the order of their content of water soluble vitamine."

APPLEMAN[17], in a recently published article, says: "Respiration in sweet corn in the milk stage is very high when the corn is first pulled. This high rate of respiratory activity falls off rapidly with storage. Catalase activity in a collateral set of ears showed a decline with storage, which is almost directly proportional to the decline in respiratory intensity after a like period of storage. The catalase activity of the expressed juice from both sweet corn and potato

[16] DUTCHER, R. ADAMS, Vitamine studies. I. Observations on the catalase activity of tissues in avian polyneuritis. Jour. Biol. Chem. **36**:63–72. 1918.

[17] APPLEMAN, C. O., Respiration and catalase activity in sweet corn. Amer. Jour. Botany **5**:207–209. 1918.

tubers is a fair index of the comparative intensity of respiration in the tissues. The data from both plant and animal tissues available at the present seem to justify the general indication that catalase action is invariably correlated with the oxidative processes involved in respiration."—WM. CROCKER.

Respiration of stored wheat.—BAILEY and GURJAR[18] have done an excellent piece of work on the respiration of stored wheat. Significant literature is well presented and related to the work in hand, and the methods used in the work are clean cut and exact. The contribution has a very important application in the shipping and storage of grains. They worked with moisture contents ranging from 12 to 18 per cent, such as appear in the practical handling of grains. The following are the more important results.

Respiration gradually and fairly uniformly rises with moisture content up to 14.5 per cent in case of plump spring wheat. With the rise of moisture above this percentage the respiration is markedly accelerated. The soft starchy wheats respire more rapidly than the hard vitreous wheats containing the same percentage of moisture. With more than 14 per cent moisture shriveled wheat respires 2 to 3 times as fast as plump wheat of the same water content, due to a larger percentage of embryo in the shriveled grains; with less than 14 per cent moisture there is little difference.

Freshly dampened wheat respires more slowly than wheat of the same water content that has been dampened for a long time or that has been naturally dampened. The difference is noticeable at 13 per cent moisture, and rises as the moisture rises. Wheat stored at room temperature respires more rapidly than that of the same moisture content at lower out-door temperatures. Unsoundness of wheat caused by the freezing of unripe plants increases respiration. This is attributed to the accumulation of glucose in the frosted grains. Increased temperature increases the respiration up to 55° C. When seeds are stored in closed chambers and the respiration taken by 4-day periods, the rate is highest for the first period and diminishes materially in successive periods as the carbon dioxide content rises. The respiration is also reduced in an oxygen free atmosphere, the ratio to that occurring in a normal atmosphere being about 1:2.5.

Many will think the author's evidence for their viscosity conception of limited respiration is insufficient. They will also question whether the amount of glucose present limits respiration when low moisture has already run respiration to so low an ebb.—WM. CROCKER.

Relation of host and parasite among fungi.—An excellent service has been rendered by REED[19] in bringing together the extensive and scattered data regarding the susceptibility of more or less related hosts to physiological strains

[18] BAILEY, C. H., and GURJAR, A. M., Respiration of stored wheat. Jour. Agric. Research **12**:685–713. 1918.

[19] REED, GEORGE M., Physiological specialization of parasitic fungi. Mem. Brooklyn Bot. Gard. **1**:348–409. 1918.

among various fungi. Some 68 species of fungi, the majority of them belonging to the Uredinales, have been reported to show such specialization. The first known and best studied species is *Puccinia graminis*, producing the destructive stem rust of wheat and of other cereals and grasses. A few species having a wide range of hosts, like *P. subnitens*, appear not to be specialized. The citation of literature includes 174 titles, supplied by 67 writers, indicating the prominence which this line of investigation has attained within the last few years. ERIKSSON's studies on the specialization of the grain rusts, reported in 1894, introduced the subject, but the fixed and unchanging character of physiological strains has first been shown definitely in the present paper, since being confirmed by STAKMAN and others.[20]

It is pointed out that so far the data do not indicate that bridging species are capable of altering the physiological nature of the parasite so as to enable it to extend the range of its natural hosts, as has heretofore been assumed. In fact, it appears that among fungous parasites there are definite strains or races not distinguishable morphologically, but only by their physiological behavior in infecting certain hosts, and that these strains retain the same characters through all the metamorphoses of the fungus, and when tested by use of any kind of reproductive body that the particular species produces. The specialization of the same fungus in widely separated regions may possibly be different, but the data are scanty. The relation of physiological specialization to morphological variation is barely mentioned. The whole subject of specialization is one of great scientific and economic interest, making the present admirable summary particularly timely.—J. C. ARTHUR.

Heath and grassland.—Continuing the investigations already noted[21] of certain English heaths and grassland, FARROW[22] has accumulated more data upon the effects of a rabbit population upon vegetation retrogression. It is demonstrated that the presence of rabbits alone is sufficient at times to change a pine forest through *Calluna* heath and *Carex arenaria* associations to a dwarf grass or a *Cladonia* heath. Experiments with irrigation and with the application of manure tend to show that both sterile soil and lack of soil moisture are factors in limiting the rate of growth and the luxuriance of the vegetation. This increased growth with improved conditions results in a decrease in the number of species in the area, since the more rapid growth of certain plants, like *Agrostis vulgaris*, smothered less vigorous ones, such as *Festuca ovina*.

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[20] STAKMAN, E. C., PARKER, J. H., and PIEMEISEL, F. J., Can biologic forms of stem rust on wheat change rapidly enough to interfere with breeding for rust resistance? Jour. Agric. Res. **14**:111–123. *pls. 13–17*. 1918.

[21] BOT. GAZ. **64**:263. 1917.

[22] FARROW, E. P., On the ecology of the vegetation of Breckland. III. General effects of rabbits on the vegetation. IV. Experiments mainly relating to the available water supply. V. Observations relating to competition between plants. Jour. Ecology **5**:1–18, 104–112, 155–172. 1917.

Evidence is also presented that such plants as *Pteris aquilina* and *Pinus* often succeed in competition owing to their dead foliage excluding the light from their competitors, causing etiolation and decay.

In a more recent paper FARROW[23] has examined the retrogression begun by rabbits and continued by sand blasts. This retrogression shows exactly the reverse order of the succession inaugurated by irrigation, being particularly noticeable in the *Agrostis vulgaris* giving place to *Festuca ovina* wherever the sand blast became intensive. Once begun, bare areas tend to increase, the sand assisting in destroying the vegetation both by direct attack and by removing the substratum, leaving clumps of grass upon the tops of small hummocks which are being constantly undermined. With the checking of wind erosion in such bare areas *Polytrichum* and *Cladonia* become agents of stabilization and revegetation.—GEO. D. FULLER.

Photosynthesis.—OSTERHOUT and HAAS[24] summarize as follows a piece of work on the dynamics of photosynthesis. "*Ulva* which has been kept in the dark begins photosynthesis as soon as it is exposed to sunlight. The rate of photosynthesis steadily increases until a constant speed is attained. This may be explained by assuming that sunlight decomposes a substance whose products catalyze photosynthesis or enter directly into the reaction. Quantitative theories are developed to account for the facts." The rate of photosynthesis was determined by the rate at which a portion of *Ulva* rendered sea water basic to phenolphthalein. Since the dissociation of carbonic acid is very slight, change of reaction is a very crude way of measuring the amount present. There is also the possibility of other exchanges of more strongly dissociating materials that could modify the reaction of the water. In the face of excellent and very accurate methods for the quantitative determination of carbon dioxide it seems hardly justifiable to use this questionable method for a study of either respiration or photosynthesis. It is also doubtful whether sufficient regard has been given to other possible limiting factors of the rate of photosynthesis in these experiments. If, in spite of the defects of experimentation, the general conclusion proves true, it is a contribution of great significance and aids in confirming WILLSTÄTTER'S view that the presence of a catalyzer is a common internal limiting factor to the rate of photosynthesis.—WM. CROCKER.

Organic plant poisons.—BRENCHLEY[25] finds hydrocyanic acid very toxic to pea and barley seedlings in water cultures. Hydrocyanic acid in concentrations of 1 part to 100,000 proved rather quickly fatal for peas and somewhat

[23] FARROW, E. P., On the ecology of the vegetation of Breckland. V. Characteristic bare areas and sand hummocks. Jour. Ecology 6:144–152. 1918.

[24] OSTERHOUT, W. J. V., and HAAS, A. R. C., Dynamical aspects of photosynthesis. Proc. Nat. Acad. Sci. 4:85–91. 1918.

[25] BRENCHLEY, WINIFRED E., Organic plant poisons. I. Hydrocyanic acid. Ann. Botany 31:447–456. 1917.

less toxic for barley. Dilutions as great as 1 part to 4,000,000 to 10,000,000 proved somewhat toxic. Hydrocyanic acid showed no stimulation and the cyanogen radicle is the toxic agent.

BRENCHLEY[26] has also studied the effect of various phenols (phenol o-cresol, m-cresol, p-cresol, resorcinol, pyrocatechol, pyrogallol, phloroglucin, orcinol) upon the growth (as indicated by increased dry weight) of barley and peas in water cultures. The purpose was to learn the direct effects of these phenols on the plants, so that it could be considered in using the phenols as partial soil sterilizers. The following concentrations were used: M/100, M/100×1/5, M/100×1/5², and M/100×1/6². The general physiological effect was the same for all the phenols, but the concentration at which these effects showed varied considerably with the different members. The highest concentration was quickly fatal with all the phenols, and the next to highest concentration with o-cresol, pyrocatechol, and pyrogallol, but there was a slight recovery in the others. The lowest concentration showed no injury in any. None of the solutions showed any stimulus effect in any concentrations.—WM. CROCKER.

Regeneration in Phegopteris.—Miss BROWN[27] has recorded the results of some experiments on regeneration in *Phegopteris polypodioides*. Near the base of the petiole of a detached leaf regeneration took place in contact with sand moistened with Knop's solution in moist air. A prothallium-like growth appeared, and from this were developed rhizoids, structures intermediate between leaves and prothallia, and true leaves. The possible determining factors are enumerated, and among them the separation of the leaf from the parent body was evidently necessary; at least it seems evident that "some phase of nutrition must be an important factor in regeneration, if not the most important factor."—J. M. C.

Selaginella.—VAN ESELTINE[28] has begun a series of contributions dealing with the American species of *Selaginella* allied to *S. rupestris*. The group is in need of critical revision, and the results will be of interest to the morphologist as well as the taxonomist. The first paper deals with the representatives of the group occurring in the Gulf Coastal Plain and the territory immediately adjacent to the northeast. In this region 8 such species are recognized, 2 of which are described as new, and an additional one was described by the same author recently. The numerous drawings and photographic plates supplement well the full descriptions.—J. M. C.

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[26] BRENCHLEY, WINIFRED E., Organic plant poisons. II. Phenols. Ann. Botany **32**:259–278. 1918.

[27] BROWN, ELIZABETH W., Regeneration in *Phegopteris polypodioides*. Bull. Torr. Bot. Club **45**:391–397. *figs.* 3. 1918.

[28] VAN ESELTINE, G. P., The allies of *Selaginella rupestris* in the southeastern United States. Contrib. U.S. Nat. Herb. **20**:159–172. *pls.* 15–22. *figs.* 63–70. 1918.

Vegetation of a glacial plunge basin.—In certain rock basins of glacial origin near Syracuse, New York, low soil and air temperatures prevail throughout the year, the difference between the rim and bottom of the depressions often amounting to 30° F. These temperature depressions have been shown by PETRY[29] to be the controlling factors in the development of plant associations characterized by distinctly northern species, such as *Cornus canadensis*, *Pyrola asarifolia*, *Coptis trifolia*, and *Ribes lacustre*, whose local distribution coincide exactly with areas of low soil and air temperature.—GEO. D. FULLER.

Geotropism and phototropism.—VAN AMEIJDEN[30] finds that neither geo-perception nor photo-perception or reaction occurs in the seedlings of *Avena sativa* or *Sinapis alba* in complete absence of oxygen. Contrary to CORRENS and KENKEL, he finds that, on complete or partial withdrawal of oxygen, the reaction of seedlings to a geotropic stimulus does not differ from their reaction to a phototropic stimulus.—WM. CROCKER.

Rusts of Costa Rica.—ARTHUR[31] has studied the rusts of Costa Rica based chiefly upon collections made by HOLWAY, and this first presentation of Costa Rican rusts includes 118 species, 22 of which are described as new, and 12 others are new to North America. The indications are that the rust flora of Costa Rica will be found to be of exceptional richness and importance.—J. M. C.

Aquilegia.—PAYSON[32] has published a revision of the North American species of *Aquilegia*. In addition to the keys, descriptions, and discussions, there is an unusually full list of stations. He recognizes 25 species, 3 of which are described as new, and also 9 subspecies or varieties, 2 of which are new.—J. M. C.

New African plants.—MOORE,[33] in connection with his studies of African Compositae, has described a new genera (*Emiliella*) of the Senecionidae and 8 new species of *Senecio*.—J. M. C.

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[29] PETRY, LOREN C., Studies of the vegetation of New York State. II. The vegetation of a glacial plunge basin and its relation to temperature. Bull. Torr. Bot. Club **45**:203–210. 1918.

[30] VAN AMEIJDEN, U. P., Geotropism and phototropism in the absence of free oxygen. Recueil Trav. Bot. Neerl. **14**:149–218. *pls. 15–19. fig. 1.* 1917.

[31] ARTHUR, J. C., Uredinales of Costa Rica based on collection by E. W. D. HOLWAY. Mycologia **10**:111–154. 1918.

[32] PAYSON, EDWIN BLAKE, The North American species of *Aquilegia*. Contrib. U.S. Nat. Herb. **20**:133–157. *pls. 8–14.* 1918.

[33] MOORE, SPENCER LEM., Alabastra diversa. Part XXIX. Jour. Botany **56**: 225–233. 1918.